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(54) Title: NOVEL CYCLOOXYGENASE VARIANTS AND METHODS OF USE

(57) **Abstract:** The invention relates to the isolation of novel cyclooxygenase type 1 (COX-l) variant enzymes. More specifically, the invention relates to the identification of cyclooxygenase transcripts harboring inton 1, or fragment thereof, of cyclooxygenase 1. The invention further relates to the diagnosis of aberrant cyclooxygenase type 1 variant gene or gene product; the identification, production, and use of compounds which modulate cyclooxygenase type 1 variant gene expression or the activity of the cyclooxygenase type 1 variant gene product including but not limited to nucleic acid encoding cyclooxygenase type 1 variants and homologues, analogues, and deletions thereof, as well as antisense, ribozyme, triple helix, antibody, and polypeptide molecules as well as small inorganic molecules; and pharmaceutical formulations and routes of administration for such compounds.

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Novel Cyclooxygenase Variants and Methods of Use

TECHNICAL FIELD

[0001] This invention relates to novel nucleic acids encoding novel mammalian cyclooxygenase (Cox) polypeptides and methods of use therefore. The invention further relates to compounds that modulate Cox activity and methods of identifying such compounds.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority from U.S. Provisional Application Serial No. 60/326,133, filed September 28, 2001, and from U.S. Provisional Application Serial No. 60/373,225, filed April 15, 2002, and from U.S. Provisional Application Serial No. 60/373,661, filed April 16, 2002, and from U.S. Provisional Application Serial No. 60/411,575, filed September 16, 2002, which are incorporated herein by reference.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0003] This invention was made with government support under grant AR46688 awarded by the National Institutes of Health (NIH). The U.S. Government may have an interest in the subject matter of this patent application.

BACKGROUND

[0004] In eukaryotic cells, polyunsaturated fatty acids are oxygenated by three general systems: 1) cyclooxygenases (COXs) and related fatty acid oxygenases, including pathogen-inducible oxygenases (PIOXs) identified in plants, animals and bacteria; 2) lipoxygenases; and 3) cytochrome P-450. Presently there are 2 COX isozymes known, COX-1 and COX-2. The predicted amino acid sequences of COX-1 cloned in chicken and mammals possesses approximately 60% amino acid sequence identity with COX-2.

[0005] The cyclooxygenation of arachidonic acid, catalyzed by two forms of cyclooxygenase produces prostaglandins which, in turn, regulate neurotransmission and immune and inflammatory responses by activating receptors coupled to cAMP formation. (Goetzl et al., FASEB J., 9:1051, 1995). For example, inflammation is both initiated and maintained, at least in part, by the overproduction of prostaglandins in injured cells. The central role that prostaglandins play in inflammation is underscored by the fact that those aspirin-like non-steroidal anti-inflammatory drugs (NSAIDS) that are most effective in the

therapy of many pathological inflammatory states all act by inhibiting prostaglandin synthesis. NSAIDs are analgesic/antiinflammatory/antipyretic medications that act as inhibitors of the cyclooxygenase active site of COX isozymes. Important mechanistic differences in the actions of individual NSAIDs with the COX active site exist. Of the NSAIDs in medical use, only aspirin is a covalent modifier of COX-1 and COX-2.

[0006] There is increasing emphasis on the development of compounds that modulate cyclooxygenase activity and methods for identifying such compounds. Therefore, there is a need for improved methods to study the effectiveness of existing anti-inflammatory drugs and to evaluate the effectiveness of potential anti-inflammatory agents, at the molecular level, as well as for reagents for use in such methods.

SUMMARY

[0007] The present invention is based, at least in part, on the discovery of novel nucleic acid molecules and polypeptides encoded by such nucleic acid molecules, referred to herein as cyclooxygenase type 1 (COX-1) variant proteins. A COX-1 variant nucleic acid molecules include those derived from the COX-1 genomic sequence and possessing intron 1. Similarly, a COX-1 variant amino acid sequence is encoded by a COX-1 variant nucleic acid sequence containing intron 1. COX-3 (i.e., pCox-1), PCOX-1a (i.e., pCox-1Δ657) or PCOX-1b are examples of COX-1 variants encompassed by the invention. Further COX-1 variants include hCOX-3(cc) (human COX-3 derived from cerebral cortex), hCOX-3(af) (human COX-3 derived from lung cells), hCOX-3 (del10) (human COX-3 derived from lung cells with exon 10 deleted) and hCOX-3(cs) (human COX-3 consensus sequence). Such variants are useful for identifying compounds or agents that modulate the activity of a COX-1 variant. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding COX-1 variant proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of COX-1 variant-encoding nucleic acids.

[0008] In one embodiment, a COX-1 variant nucleic acid molecule of the invention comprises intron 1, or fragment thereof, of cycloxygenase type 1. In one aspect, the nucleic acid molecule is an mRNA transcript. In another aspect, the nucleic acid molecule is cDNA. In another aspect, the nucleic acid molecule encodes a polypeptide comprising at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, and at least one heme binding domain. In yet another aspect the

nucleic acid molecule encodes a cyclooxygenase polypeptide, or naturally occurring allelic variant thereof, which comprises intron 1, or fragment thereof, of cyclooxygenase 1.

[0009] In another embodiment, the invention provides an isolated COX-1 variant polypeptide comprising an amino acid sequence encoded by intron 1, or fragment thereof, of cyclooxygenase 1, wherein the polypeptide catalyzes the oxygenation and/or cyclization of a fatty acid. In one aspect, the isolated polypeptide further comprises at least one membrane-binding domain and at least one heme binding domain.

[0010] In another embodiment, a COX-1 variant nucleic acid molecule of the invention is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1, SEQ ID NO:3 (i.e., COX-3) or a complement thereof. In another embodiment, a COX-1 variant nucleic acid molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% homologous to a nucleotide sequence including SEQ ID NO:4, SEQ ID NO:6 (i.e., PCOX-1a), or a complement thereof. In another embodiment, a COX-1 variant nucleic acid molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% homologous to a nucleotide sequence including SEQ ID NO:7, SEQ ID NO:9 (i.e., PCOX-1b), or a complement thereof. In another embodiment, a COX-1 variant nucleic acid molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% homologous to a nucleotide sequence including SEQ ID NO:10 (i.e., hCOX-3(cc)), or a complement thereof. In another embodiment, a COX-1 variant nucleic acid molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% homologous to a nucleotide sequence including SEQ ID NO:11 (i.e., hCOX-3(af), or a complement thereof. In another embodiment, a COX-1 variant nucleic acid molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% homologous to a nucleotide sequence including SEQ ID NO:12 (i.e., hCOX-3(del10)), or a complement thereof. In yet another embodiment, a COX-1 variant nucleic acid molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% homologous to a nucleotide sequence including SEQ ID NO:13 (i.e., hCOX-3(cs)), or a complement thereof.

[0011] In one embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:1 or 3, or a complement thereof. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1 or 3.

[0012] In another embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:4 or 6, or a complement thereof. In another preferred embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:4 or 6.

[0013] In yet another embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:10, or a complement thereof. In another embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:10.

[0014] In yet another embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:11, or a complement thereof. In another embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:11.

[0015] In yet another embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:12, or a complement thereof. In another embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:12.

[0016] In yet another embodiment, the isolated nucleic acid molecule includes the nucleotide sequence shown SEQ ID NO:13, or a complement thereof. In another embodiment, the nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:13.

[0017] In another embodiment, a COX-1 variant nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having an amino acid sequence homologous to the amino acid sequence of SEQ ID NO:2 (i.e., COX-3), SEQ ID NO:5 (i.e., PCOX-1a), SEQ ID NO:14 (hCOX-3(cs1), SEQ ID NO:15 (i.e., hCOX-3(cs2) and SEQ ID NO:16 (i.e., hCOX-3(cs3). In a one embodiment, a COX-1 variant nucleic acid molecule includes a nucleotide sequence encoding a polypeptide having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to an amino acid sequence including SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16 (e.g., the entire amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16).

[0018] In one embodiment, an isolated nucleic acid molecule encoding the amino acid sequence of a COX-1 variant is derived from a mammalian source, for example, ovine, porcine, lupine or canine. In another embodiment, a COX-1 variant is derived from chicken. In yet another embodiment, a COX-1 variant is derived from human.

[0019] Another embodiment of the invention features nucleic acid molecules, preferably COX-1 variant nucleic acid molecules, which specifically detect COX-1 variant nucleic acid molecules encoding a COX-1 variant polypeptide relative to nucleic acid molecules encoding non-COX-1 variant polypeptides. For example, in one embodiment, such a nucleic acid molecule is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750,

or 800 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, or a complement thereof.

[0020] In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide which includes the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule which includes SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13 under stringent conditions.

[0021] Another embodiment of the invention provides an isolated nucleic acid molecule that is antisense to a COX-1 variant nucleic acid molecule, e.g., the coding strand of a COX-1 variant nucleic acid molecule.

[0022] Another aspect of the invention provides a vector comprising a COX-1 variant nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. The invention also provides a method for producing a protein or polypeptide, preferably a COX-1 variant protein or polypetide, by culturing in a suitable medium, a host cell, e.g., a mammalian host cell or insect cell, of the invention containing a recombinant expression vector, such that the protein is produced.

[0023] Another aspect of this invention features isolated or recombinant COX-1 variant proteins and polypeptides. In one embodiment, the isolated protein, preferably a COX-1 variant protein (e.g., COX-3, PCOX-1a, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs)), includes at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, and at least one heme binding domain. In another embodiment, the isolated protein, preferably a COX-1 variant protein, includes at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, at least one heme binding domain, and has an amino acid sequence which is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to an amino acid sequence including SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16. In yet another embodiment, the isolated protein, preferably a COX-1 variant protein, includes at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, at least one heme binding domain, and is expressed and/or functions in cells of the central nervous system. In an even further embodiment, the isolated protein,

preferably a COX-1 variant protein, includes at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, at least one heme binding domain and plays a role in signaling pathways associated with cellular growth, e.g., signaling pathways associated with cell cycle regulation and central nervous system function. In another embodiment, the isolated protein, preferably a COX-1 variant protein, includes at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, at least one heme binding domain, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

[0024] In another embodiment, the isolated protein has an amino acid sequence homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16. In one embodiment, the protein has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to an amino acid sequence including SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16, (e.g., the entire amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16). In another embodiment, the invention features fragments of the proteins having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16, wherein the fragment comprises at least 15 amino acids (e.g., contiguous amino acids) of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16. In another embodiment, the protein has the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

[0025] Another embodiment of the invention features an isolated protein, preferably a COX-1 variant protein, which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to a nucleotide sequence (e.g., to the entire length of the nucleotide sequence) including SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, or a complement thereof. This invention further features an isolated protein, preferably a COX-1 variant protein, which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of

SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, or a complement thereof.

[0026] The proteins of the present invention or biologically active portions thereof, can be operatively linked to a non-COX-1 variant polypeptide (e.g., heterologous amino acid sequences) to form fusion proteins. The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind proteins of the invention, preferably COX-1 variant proteins (e.g., COX-3, PCOX-1a, PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs)). In addition, the COX-1 variant proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

[0027] In another embodiment, the present invention provides a method for detecting the presence of a Cox-1 variant nucleic acid molecule, protein or polypeptide in a biological sample by contacting the biological sample with an agent capable of detecting a COX-1 variant nucleic acid molecule, protein or polypeptide such that the presence of a COX-1 variant nucleic acid molecule, protein or polypeptide is detected in the biological sample.

[0028] In another embodiment, the present invention provides a method for detecting the presence of COX-1 variant activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of COX-1 variant activity such that the presence of COX-1 variant activity is detected in the biological sample.

[0029] In another aspect the invention provides a method for identifying a compound that modulates the activity of a COX-1 variant protein or nucleic acid by providing an indicator composition comprising a COX-1 variant protein having COX-1 variant activity, contacting the indicator composition with a test compound, and determining the effect of the test compound on COX-1 variant activity in the indicator composition to identify a compound that modulates the activity of a COX-1 variant protein.

[0030] In another aspect, the invention provides a method for modulating COX-1 variant activity comprising contacting a cell capable of expressing COX-1 variant with an agent that modulates COX-1 variant activity such that COX-1 variant activity in the cell is modulated. In one embodiment, the agent inhibits COX-1 variant activity. In another embodiment, the agent stimulates COX-1 variant activity. In one embodiment, the agent is an antibody that specifically binds to a COX-1 variant protein. In another embodiment, the agent modulates expression of a COX-1 variant by modulating transcription of a COX-1 variant gene or translation of a COX-1 variant mRNA. In yet another embodiment, the agent is a nucleic

acid molecule having a nucleotide sequence that is antisense to the coding strand of a COX-1 variant mRNA or a COX-1 variant gene.

[0031] In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant COX-1 variant protein or nucleic acid expression or activity by administering an agent that is a COX-1 variant modulator to the subject. In one embodiment, the COX-1 variant modulator is a COX-1 variant protein. In another embodiment the COX-1 variant modulator is a COX-1 variant nucleic acid molecule. In yet another embodiment, the COX-1 variant modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant COX-1 variant protein or nucleic acid expression is a cellular growth related disorder, e.g., a neoplastic disorder, or a disorder of the central nervous system, e.g., Alzheimer's Disease.

[0032] The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a COX-1 variant protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a COX-1 variant protein, wherein a wild-type form of the gene encodes a protein with a COX-1 variant activity. A diagnostic assay can include, for example, an array-based system for detecting the presence or absence of a COX-1 variant or the presence or absence of a genetic alteration in a COX-1 variant.

[0033] In another embodiment, the invention provides a method for ameliorating a neurodegenerative condition in a subject by administering a specific inhibitor of a COX-1 variant encoded by a nucleic acid as set forth in the present invention, in a pharmaceutically acceptable carrier.

[0034] In another embodiment, the invention provides a method for selectively inhibiting COX-3, PCOX-1a, PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) activity in a subject by administering a compound that selectively inhibits activity of the COX-3, PCOX-1a, PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) gene product to a subject in need of such treatment.

[0035] In another embodiment, the invention provides a method for selectively inhibiting COX-3, PCOX-1a, PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) activity in a subject by administering a non-steroidal compound that selectively inhibits activity of the COX-3, PCOX-1a, PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) gene product in a subject in need of such treatment, wherein the activity of the non-steroidal compound does not result in significant toxic side effects in the subject.

[0036] In yet another embodiment, the invention provides a method for selectively inhibiting COX-1 variant activity in a subject by administering a non-steroidal compound that selectively inhibits activity of a COX-1 variant gene product in a subject in need of such treatment, wherein the ability of the non-steroidal compound to selectively inhibit the activity of the COX-1 variant gene product is determined by contacting a genetically engineered cell that expresses, for example, COX-3, PCOX-1a, PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs), and not COX-1 or COX-2, with the compound and exposing the cell to a pre-determined amount of arachidonic acid; contacting a genetically engineered cell that expresses COX-1 or COX-2, and not a COX-1 variant, with the compound and exposing the cell to a pre-determined amount of arachidonic acid; measuring the conversion of arachidonic acid to its prostaglandin metabolite; and comparing the amount of the converted arachidonic acid converted by each cell exposed to the compound to the amount of the arachidonic acid converted by control cells that were not exposed to the compound, so that the compounds that inhibit a COX-1 variant activity and not COX-1 or COX-2 activity are identified.

[0037] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0038] Figure 1A depicts an alignment of known COX-2 sequences. "Guinea" represents guinea pig, "Rainbow" is rainbow trout, and "Brook" is brook trout. Asterisks (*) are placed above cysteine residues that are known to be involved in disulfide bonding and gaps in the sequence are represented by a dash (-). All numbering in these alignments uses the ovine sequence as a reference. Domains are indicated.

[0039] Figure 1B depicts an alignment of known COX-1 amino acid sequences for human (SEQ ID NO:22), ovine (SEQ ID NO:23), canine (SEQ ID NO:24), bovine (SEQ ID NO:25), equine (SEQ ID NO:26), rabbit (SEQ ID NO:27), guinea (SEQ ID NO:28), murine (SEQ ID NO:29), rat (SEQ ID NO:30), mink (SEQ ID NO:30), chicken (SEQ ID NO:31), rainbow trout (SEQ ID NO:32), and brook trout (SEQ ID NO:33). Asterisks, dashes and alignment are the same as those indicated for Figure 1A. Domains are indicated.

[0040] Figure 1C depicts an alignment of the consensus amino acid sequences of COX-1 (SEQ ID NO:34) and COX-2 (SEQ ID NO:35) with the sequences of cyclooxygenases from *Gersemia fruticosa* (SEQ ID NO:36) and *Plexaura homomalla* (SEQ ID NO:37), which are

two species of coral. A period represents a residue for which no clear consensus (50% or greater) could be seen.

[0041] Figure 2 depicts the primary sequence alignment of the consensus cyclooxygenase sequences with three plant PIOXs isolated from rice (SEQ ID NO:38), A. Thaliana (SEQ ID NO:39), and tobacco (SEQ ID NO:40). The domains shown are for cyclooxygenase. Periods represent residues for which there is no consensus (less than 50% of known sequences have the same residue here). The signal peptide and EGF-like, binding, and dimerization domains are not present in the PIOX sequences. Additionally, the distal histidine is not present in the PIOX sequences. The "REHN" sequence is part of the peroxidase catalytic domain of cyclooxygenases, and represents a stretch of 9 amino acids that is absolutely conserved in all known cyclooxygenases. This sequence is degenerated in the PIOXs. The active site tyrosine and proximal histidine heme ligand are conserved in all of the PIOX sequences.

[0042] Figure 3 depicts activation of arachidonic acid by COX enzymes showing the essential role of Tyr385– an amino acid residue conserved in COXs and PIOXs. Step 1: Arachidonate is coordinated in the COX active site in an extended L shape, its carboxyl group being coordinated by ARG120 and TYR355. The Pro-S hydrogen from carbon 13 is abstracted by Tyr385 to form an arachidonyl radical. Step 2: Oxygen presumed to have diffused in to the COX active site through the mouth of the channel attacks the arachidonyl radical forming an endoperoxyl and cyclopentane ring. Step 3: The endoperoxyl radical is attacked by a second molecule of oxygen at carbon 15. Step 4: Tyr385 donates its hydrogen to form PGG2 and to reform the radical at Tyr385.

[0043] Figure 4 depicts a diagram of the catalytic cycle responsible for activation of Tyr^{385} – an amino acid residue conserved in COXs and PIOXs. An endogenous oxidant binds the peroxidase active site and oxidizes ferric heme to ferryl-oxo-porphyrin radical which in turn abstracts hydrogen from Tyr^{385} to form a tyrosyl radical. Only one oxidation event at the peroxidase is needed to activate the enzyme, since the Tyr^{385} radical is regenerated with each cyclooxygenase cycle.

[0044] Figure 5, panel A, depicts Northern blot analysis of the distribution of COX-1 in dog tissues. Arrows indicate additional COX-1 transcripts in brain tissue. S = stomach; d = duodenum; i = ileum; j = jejunum; c = colon; l = liver; s = spleen; b = brain; lu = lung; o = ovary; k = kidney; m = MDKC cells (ind). The blots were hybridized with ^{32}P-labeled canine COX-1 DNA (specific activity 8.8×10^8cpm/µg ,6×10^6cpm/ml). Post hybridization washes were carried out for 3h with 3-4 changes in 2 x SSC/0.5%SDS at about 65°C.

[0045] Figure 5, panel B,, depicts Northern blot analysis of the distribution of COX-1 in chicken tissues. Arrows indicate additional COX-1 transcripts in brain tissue. v = seminal vesicle; p = pancreas; t = testicle; h = heart; ly = bursal lymph. The blots were hybridized as described for Figure 5, panel a).

[0046] Figure 5, panel C, depicts a Northern blot analysis of PCOX-1 (i.e., COX-3) and PCOX-1Δ657 (i.e., PCOX1a) RNA.

[0047] Figure 6A depcits a nucleic acid sequence alignment of canine COX-1, PCOX-1 (i.e., COX-3) and PCOX-1Δ657 (i.e., PCOX1a) and a consensus sequence.

[0048] Figure 6B depicts the predicted amino acid sequence of canine COX-1, PCOX-1 (i.e., COX-3) and PCOX-1Δ657 (i.e., PCOX1a) as compared with a consensus sequence.

[0049] Figure 7 depicts an analysis of the structure of intron-1 of a COX-1 variant in human (SEQ ID NO:41) and mouse (SEQ ID NO:42) as compared with canine (SEQ ID NO:43). The human and mouse sequences contain an intron-1 that is similar in size to that in canine which, when retained, would provide an in-frame insertion into the signal peptide encoding the protein.

[0050] Figure 8 depicts a Northern blot analysis of canine total RNA (25ug) probed with ^{32}P-end labeled oligo (50mer) designed from within the first intron of COX-1 in canine. The blot was washed at a final stringency of Tm-4 (Tm = calculated melting temperature). S = stomach; D = duodenum; I = ileum; J = jejunum; C = colon; L = liver; Sp = spleen; Bm = brain (cerebral cortex)- mRNA (2.5ug); Bt = brain (cerebral cortex)- total RNA; Lu = lung; O = ovary; K = kidney; Cl = CCl 34 cells. The 5.2 kb mRNA that was detected in panels A,B and C and is designated by an arrow in Figure 8.

[0051] Figure 9A depicts the nucleotide coding sequence of COX-3.

[0052] Figure 9B depicts the amino acid sequence of a COX-3 polypeptide.

[0053] Figure 9C depicts the cDNA sequence of COX-3 transcript.

[0054] Figure 9D depicts the nucleotide coding sequence of PCOX-1a.

[0055] Figure 9E depicts the amino acid sequence of PCOX-1a polypeptide.

[0056] Figure 9F depicts the cDNA sequence of PCOX-1b transcript.

[0057] Figure 10, panel A, depicts a Northern blot analysis and RT-PCR of canine cerebral cortex poly(A) RNA (lane 1, 5.0 μg; lane 2, 2.5 μg) probed with 1)^{32}P-labeled canine COX-1 cDNA fragment, 2) ^{32}P-labeled canine antisense oligonucleotide to intron 1 (CCI).

[0058] Figure 10, panel B, depicts PCR amplification of PCOX-1 in canine cerebral cortex. Lane 1, ethidium bromide-stained gel of amplified products corresponding to PCOX-1a containing intron 1 (upper band) and PCOX-1b (lower band) lacking intron 1; lane 2,

Southern blot of the amplified products probed with antisense oligonucleotide (CCI) to intron 1; and lane 3, Southern blot using COX-3 cDNA as probe.

[0059] Figure 10, panel C, depicts human Multiple Tissue Northern blots (MTNR) probed with a ^{32}P-labeled human antisense oligonucleotide to intron 1 (HCI). The ~5.2 kb mRNA was detected in blots 1-3 (adult tissues), and 4 (fetal tissues). Abbreviations: Am, amygdala; B, brain; C, cerebellum: Cc, cerebral cortex; Fl, frontal lobe; H, hippocampus; Ht, heart; K, kidney; L, lung; Li, liver; M, skeletal muscle; Md, medulla; N, caudate nucleus; Op, occipital pole; P, placenta; Pn, pancreas; Pu, putamen; Sc, spinal cord; T, thalamus; Tl, temporal lobe; X, corpus callosum.

[0060] Figure 11 depicts a schematic representation of the domains of COX-3 and PCOX-1 in comparison to COX-1. Abbreviations: s, signal peptide; d1, dimerization domain/EGF-like domain 1; d2, dimerization domain 2; m, membrane binding domain; c, catalytic domain; i, 90 bp sequence encoded by intron 1.

[0061] Figure 12 depicts Western blots showing the expression of COX-3, PCOX-1a and COX-1 in insect cells treated with (+) and without (-) tunicamycin (top panels). Arrows indicate glycosylated forms of COX-1 which are not present in cells treated with tunicamycin. Polyclonal antibodies to human and mouse COX-1 intron 1 sequence were used to probe the COX-3 and PCOX-1a blots while a monoclonal antibody to ovine COX-1 (Cayman) was used to probe the mouse COX-1 blot. COX activity in insect cells expressing COX-3, PCOX-1a, and COX-1. Cells were treated with (+) and without (-) tunicamycin (bottom panels).

[0062] Figure 13 depicts line-graphs of drug-inhibition-studies showing the effects of acetaminophen (panel A and panel B), phenacetin (panel C), and dipyrone (panel D) on COX-1 (◆), COX-2 (●) and COX-3 (■) activity in insect cells. COX activity was measured by the formation of PGE_2 after exposure to exogenous 5 μM (A) or 30 μM (B, C, D) arachidonic acid for 10 minutes. Data are expressed as mean ±SEM (n= 6-9).

[0063] Figure 14 depicts an alignment of the consensus sequences of COX-1, COX-2 and coral COX corresponding to structural helices H2, H5, and H8 with plant PIOXs and LDS. The PIOXs are from *Oryza sativa* (rice), *Arabidopsis thaliana* (arabidopsis) and *Nicotiana tabacum* (tobacco). Also aligned is linoleate diol synthase from *Gaeumannomyces graminis* (LDS). The consensus is shown using small letters for >50% homology and capital letters for 100% homology. Periods indicate that <50% homology was seen.

[0064] Figure 15, panel A, depicts a Western blot of human aorta lysate probed with COX-1 and COX-3 antibodies. The blot (lanes 3-8, 20 μg total aorta protein each lane) probed with primary, secondary, or blocked antibodies as indicated. A solid horizontal arrow indicates the 65 kDa protein, an open arrow indicates the 53 kDa proteins, and an upward diagonal solid arrow indicates the 50 kDa protein. A single asterisk (*) denotes unglycosylated canine COX-3, and a double asterisk (**) denotes unglycosylated canine PCOX-1a.

[0065] Figure 15, panel B, depicts a densitometric analysis of 65, 53, and 50 kDa proteins. Percent relative densitometric units (% rdu) were calculated by comparison to the signal from unblocked primary antibodies. The 50kDa protein is not detected (n/d) by unblocked or blocked COX-3 PAb.

[0066] Figure 16 depicts a comparison of hCOX-3(cc), hCOX-3(af) and hCOX-3(del10) cDNA and provides a hCOX-3(cs) consensus sequence (SEQ ID NO:13) which is a consensus sequence of hCOX-3(af), hCOX-3(cc) and hCOX-3(del10).

[0067] Figure 17 depicts the cDNA sequence of hCOX-3(cc) transcript (SEQ ID NO:10).

[0068] Figure 18 depicts the cDNA sequence of hCOX-3(af) transcript (SEQ ID NO:11).

[0069] Figure 19 depicts the cDNA sequence of hCOX-3(del10) transcript (SEQ ID NO:12).

[0070] Figure 20A-F depicts consensus amino acid sequences (SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16) for different reading frames of hCOX-3(cs) (SEQ ID NO:13).

[0071] Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

[0072] The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as "cyclooxygenase type 1 variants, " "COX-1 variants" or "COX-1 variant nucleic acid and polypeptide molecules," which play a role in or function in signaling pathways associated with cell processes in brain and other tissues. Exemplary COX-1 variants of the invention include COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs). In one embodiment, the COX-1 variant molecules modulate the activity of one or more proteins involved in cellular growth or differentiation. In another embodiment, the COX-1 variant molecules of the present invention are capable of modulating the function of the central nervous system.

[0073] Cyclooxygenase isozymes are the cellular targets of non-steroidal antiinflammatory drugs (NSAIDs), which include pharmaceutically-important therapeutic

agents such as aspirin, ibuprofen, and naproxen. The present invention provides novel COX-1 variant enzymes. Fatty acid oxygenase activity is central to the production of prostaglandins, thromboxanes, hydroxy- and hydroperoxy-fatty acids by cyclooxygenases and is also shared by a related group of enzymes, which in plants are called pathogen inducible fatty acid oxygenases (PIOXs). The present data indicate that PIOX-like enzymes are found widely in nature. PIOXs make hydroperoxy-fatty acids and their derivatives. Thus, the present COX-1 variants, like PIOXs, contain the critical amino acid residues needed to synthesize important oxygenated fatty acid-derived messengers in the brain and in other tissue.

[0074] As previously noted, cyclooxygenases play a role in prostaglandin synthesis. Inhibition or over stimulation of the activity of cyclooxygenases involved in signaling pathways associated with cellular growth can lead to perturbed cellular growth, which can in turn lead to cellular growth related disorders. As used herein, a "cellular growth related disorder" includes a disorder, disease, or condition characterized by a deregulation, e.g., an upregulation or a downregulation, of cellular growth. Cellular growth deregulation may be due to a deregulation of cellular proliferation, cell cycle progression, cellular differentiation and/or cellular hypertrophy. Examples of cellular growth related disorders include disorders such as cancer, e.g., melanoma, prostate cancer, cervical cancer, breast cancer, colon cancer, or sarcoma. Cellular growth related disorders further include disorders related to unregulated or dysregulated apoptosis (i.e., programmed cell death). Apoptosis is a cellular suicide process in which damaged or harmful cells are eliminated from multicellular organisms. Cells undergoing apoptosis have distinct morphological changes including cell shrinkage, membrane blebbing, chromatin condensation, apoptotic body formation and fragmentation. This cell suicide program is evolutionarily conserved across animal and plant species. Apoptosis plays an important role in the development and homeostasis of metazoans and is also critical in insect embryonic development and metamorphosis. Furthermore, apoptosis acts as a host defense mechanism. For example, virally infected cells are eliminated by apoptosis to limit the propagation of viruses. Apoptosis mechanisms are involved in plant reactions to biotic and abiotic insults. Dysregulation of apoptosis has been associated with a variety of human diseases including cancer, neurodegenerative disorders and autoimmune diseases. Accordingly, identification of novel mechanisms to manipulate apoptosis provides new means to study and manipulate this process.

[0075] The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as COX-1 variant protein and nucleic acid molecules, which

comprise a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologues of non-human origin. Members of a family may also have common functional characteristics. One embodiment of the invention features exemplary COX-1 variants including COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs). The nucleic acid and protein molecules of the invention are described in further detail in the following subsections.

[0076] In one embodiment, a COX-1 variant nucleic acid molecules of the invention comprises intron 1, or fragment thereof, of cycloxygenase type 1. In one aspect, the nucleic acid molecule is an mRNA transcript. In another aspect, the nucleic acid molecule is cDNA. In another aspect, the nucleic acid molecule encodes a polypeptide comprising at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, and at least one heme binding domain. In yet another aspect the nucleic acid molecule encodes a cyclooxygenase polypeptide, or naturally occurring allelic variant thereof, which comprises intron 1, or fragment thereof, of cyclooxygenase 1.

[0077] The present invention provides nucleic acid molecules and polypeptides of COX-1 variants that possess a cyclooxygenase intron-1. Retention of intron-1 in these mRNA transcripts and cDNAs disrupts the signal peptide of the protein and, therefore, is predicted to change the subcellular localization of the proteins encoded by these mRNA transcripts and any cDNA derived therefrom. Changing the subcellular localization of these proteins from the lumen of the endoplasmic reticulum, where COX-1 is normally found, to the cytosol or other location will change posttranslational modifications, redox states, and protein-protein interactions of the proteins encoded by our cDNAs. These changes are predicted to have significant effect on the enzymatic activities of the proteins that will make them novel drug targets separate from COX-1.

[0078] Northern blot and RT-PCR data provided herein suggest that analogous sequences exist in humans. These data show that human tissues contain mRNAs encoded from the COX-1 gene that possess intron-1 sequences. Although cyclooxygenase intron-1 has been previously sequenced, the inventions disclosed herein provide the first evidence that intron-1

sequences are contained in mature cyclooxygenase transcripts. Alignment of these sequences shows that in each species intron one is short (90-102nt), the length of its sequence is a multiple of three, and the sequence is evolutionarily conserved. Because the sequence is a multiple of three, intron-1 constitutes an in-frame insertion when the sequence is retained in a COX-1 mRNA. The evolutionary conservation of the sequence predicts a conserved sequence encoded by the 5' end of the intron that is present in all 3 species and may be important in subcellular targeting of the protein.

[0079] An exemplary COX-1 variant includes COX-3. Northern blot analysis of (polyA) RNA from human tissues using an anti-sense oligonucleotide probe to intron 1 shows that a ~5.2 kb mRNA and, in some cases smaller mRNAs, are specifically detected by this probe. Expression of this human ~5.2 kb, intron-1-hybridizing RNA was highest in brain cortex, the same tissue which possesses a high amount of intron-1 containing COX-1 mRNA in dog. The ~5.2 kb RNA, from which COX-3 is expressed, was also found to be present in other parts of the brain and in other tissues such as heart and muscle. Reverse-transcriptase-coupled polymerase chain reaction (RT-PCR) using a primer pair consisting of a sense primer specific for intron-1 and an antisense primer to the region of the stop codon of the COX-1 open reading frame was performed using human brain RNA as template. This experiment produced a 1.8kb fragment, which is the correct size for amplification of human COX-1 cDNA containing intron-1. Moreover, hybridization of this blot with murine-COX-1 cDNA hybridized strongly at high stringency to the amplified fragment, demonstrating that this fragment contains human COX-1 cDNA.

[0080] In one embodiment, the isolated COX-1 variant proteins or polypeptides are identified based on the presence of at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, and at least one heme binding domain.

[0081] Isolated proteins of the present invention have an amino acid sequence homologous to the amino acid sequence of COX-3, PCOX-1a, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) or are encoded by a nucleotide sequence homologous to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13. As used herein, the term "homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs

and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains have at least 30%, 40%, or 50% homology, preferably 60% homology, more preferably 70%-80%, and even more preferably 90-95% homology across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently homologous. Furthermore, amino acid or nucleotide sequences which share at least 30%, 40%, or 50%, preferably 60%, more preferably 70-80%, or 90-95% homology and share a common functional activity are defined herein as sufficiently homologous.

[0082] As used interchangeably herein a "COX-3 activity", "biological activity of COX-3" or "functional activity of COX-3", refers to an activity exerted by a COX-3 protein, polypeptide or nucleic acid molecule on a COX-3 responsive cell or a COX-3 protein substrate, as determined in vivo, or in vitro, according to standard techniques. The biological activity of COX-3 is described herein. Similarly, "PCOX-1a activity", "biological activity of PCOX-1a" or "functional activity of PCOX-1a", refers to an activity exerted by a PCOX-1a protein, polypeptide or nucleic acid molecule on a PCOX-1a responsive cell or a PCOX-1a protein substrate, as determined in vivo, or in vitro, according to standard techniques. The biological activity of PCOX-1a is described herein. The previously described terms are applicable to all exemplary COX-1 variants described herein, including PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs).

[0083] The nucleotide sequence of the isolated COX-3 cDNA and the predicted amino acid sequence of the COX-3 polypeptide are shown in Figures 9A (SEQ ID NO:1) and 9B (SEQ ID NO:2), respectively. The nucleotide sequence of the isolated PCOX-1a cDNA and the predicted amino acid sequence of the PCOX-1a polypeptide are shown in Figures 9D (SEQ ID NO:4) and 9E (SEQ ID NO:5), respectively. The consensus nucleotide sequence of hCOX-3(cc), hCOX-3(af) and hCOX-3(del10) cDNA is shown in Figure 16 (SEQ ID NO:13). The nucleotide sequence of the isolated hCOX-3(cc) cDNA is shown in Figure 17 (SEQ ID NO:10). The nucleotide sequence of the isolated hCOX-3(af) cDNA is shown in Figure 18 (SEQ ID NO:11). The nucleotide sequence of the isolated hCOX-3(del10) cDNA is shown in Figure 19 (SEQ ID NO:12). The amino acid sequences derived from the consensus sequence is shown in Figure 20A-F (SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO16).

[0084] Plasmids containing the nucleotide sequence encoding COX-3, PCOX1a, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) were deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on

________ and assigned Accession Numbers ________. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

[0085] One embodiment of the invention pertains to isolated nucleic acid molecules that encode proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify COX-1 variants (i.e., COX-3, PCOX-1a, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) -encoding nucleic acids (e.g., mRNA) and fragments for use as PCR primers for the amplification or mutation of nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

[0086] An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a COX-1 variant cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

[0087] A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, using all or portion of the nucleic acid sequence of SEQ ID NO:1, or the nucleotide sequence of SEQ ID NO:3, as a hybridization probe, nucleic acid molecules can be isolated using standard hybridization and cloning

techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

[0088] Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, respectively.

[0089] A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to COX-1 variant nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

[0090] In various embodiments, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13 corresponding to the coding region of COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs), respectively.

[0091] In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, respectively, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, respectively, thereby forming a stable duplex.

[0092] In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 54%, 55%, 60%, 62%, 65%, 70%, 75%, 78%, 80%, 85%, 86%, 90%, 95%, 97%, 98% or more

homologous to the nucleotide sequence (e.g., to the entire length of the nucleotide sequence) shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, or a portion of any of these nucleotide sequences.

[0093] Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a protein. The nucleotide sequence determined from the cloning of the COX-1 variant transcript allows for the generation of probes and primers designed for use in identifying and/or cloning other COX-1 variant family members, as well as homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13. In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least 350, 400, 450, 500, 550, 600, 650, 700, 750, or 800 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13

[0094] As defined herein, nucleic acids that do not hybridize to each other under stringent conditions are still substantially homologous to one another if they encode polypeptides that are substantially identical to each other. This occurs, for example, when a nucleic acid is created synthetically or recombinantly using a high codon degeneracy as permitted by the redundancy of the genetic code.

[0095] Probes based on COX-1 variant nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which misexpress a COX-1 variant protein, such as by measuring a level of a COX-1 variant-

encoding nucleic acid in a sample of cells from a subject e.g., detecting COX-1 variant mRNA levels.

[0096] A nucleic acid fragment encoding a "biologically active portion of a COX-1 variant protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, which encodes a polypeptide having a biological activity (the biological activities of the proteins are described herein), expressing the encoded portion of the protein (e.g., by recombinant expression in vitro) and assessing the activity of the encoded portion of the protein.

[0097] The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, due to the degeneracy of the genetic code and, thus, encode the same proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

[0098] In addition to the COX-1 variant nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the proteins may exist within a population (e.g., the human population). Such genetic polymorphism may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a COX-1 protein, preferably a mammalian protein, and can further include non-coding regulatory sequences, and introns. Since natural allelic variations can arise in the COX-1 gene, variant mRNA transcripts encoding COX-1 variant polypeptides can include such allelic variations. Any and all such nucleotide variations and resulting amino acid polymorphisms in COX-1 variant genes that are the result of natural allelic variation and that do not alter the functional activity of a COX-1 variant protein are intended to be within the scope of the invention.

[0099] Moreover, nucleic acid molecules encoding other COX-1 family members and, thus, which have a nucleotide sequence which differs from the sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, are intended to be within the scope of the invention. For example,

another COX-1 variant cDNA can be identified based on the nucleotide sequence of the disclosed human, canine or chicken sequences. Moreover, nucleic acid molecules encoding proteins of the invention from different species, and thus which have a nucleotide sequence which differs from the disclosed sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13 are intended to be within the scope of the invention. For example, a mouse COX-1 variant cDNA can be identified based on the nucleotide sequence of a human, canine or ovine.

[00100] Nucleic acid molecules corresponding to natural allelic variants and homologues of the cDNAs of the invention can be isolated based on their homology to the COX-1 variant nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

[00101] Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, or 600 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 30%, 40%, 50%, or 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

[00102] In addition to naturally-occurring allelic variants of the sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, thereby leading to changes in the amino acid sequence of the encoded proteins, without altering the functional ability of the proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of (e.g., the sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) proteins of the present invention, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the proteins of the present invention and other family members are not likely to be amenable to alteration.

[00103] Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding proteins of the invention that contain changes in amino acid residues that are not essential for activity. Such proteins differ in amino acid sequence from SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 41%, 42%, 45%, 50%, 55%, 59%, 60%, 65%, 70%, 75%, 80%, 81%, 85%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16, (e.g., the entire amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO15 or SEQ ID NO:16).

[00104] An isolated nucleic acid molecule encoding a protein of the invention which is homologous to the protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, respectively, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ

ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a protein of the invention is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a COX-1 variant coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for COX-1 variant biological activity to identify mutants that retain activity. Following mutagenesis SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

[00105] In addition to the nucleic acid molecules encoding COX-3, PCOX-1a, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire COX-1 variant coding strand, or only to a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a COX-1 variant. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding a protein of the invention. The term "noncoding region" refers to 5' and 3'

sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

[00106] Given the coding strand sequences encoding COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[00107] The antisense nucleic acid molecules of the invention are typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[00108] In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) Nature 334:585-591)) can be used to catalytically cleave COX-1 variant mRNA transcripts to thereby inhibit translation of mRNA. A ribozyme having specificity for a -encoding nucleic acid can be designed based upon the nucleotide sequence of a COX-1 variant cDNA disclosed herein (i.e., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a -encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) Science 261:1411-1418.

[00109] Alternatively, COX-1 variant gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the (e.g., the promoter

and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene, C. (1991) Anticancer Drug Des. 6(6):569-84; Helene, C. et al. (1992) Ann. N.Y. Acad. Sci. 660:27-36; and Maher, L.J. (1992) Bioassays 14(12):807-15.

[00110] In yet another embodiment, the nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) Bioorganic & Medicinal Chemistry 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) supra; Perry-O'Keefe et al. Proc. Natl. Acad. Sci. 93: 14670-675.

[00111] PNAs of nucleic acid molecules disclosed herein can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of the present nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) supra)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) supra; Perry-O'Keefe supra).

[00112] In another embodiment, PNAs of a COX-1 variant can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of COX-1 variant nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (e.g., RNAse H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) supra). The synthesis of PNA-DNA

chimeras can be performed as described in Hyrup B. (1996) supra and Finn P.J. et al. (1996) Nucleic Acids Res. 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. et al. (1989) Nucleic Acid Res. 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. et al. (1996) supra). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. et al. (1975) Bioorganic Med. Chem. Lett. 5: 1119-11124).

[00113] In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) Proc. Natl. Acad. Sci. US. 86:6553-6556; Lemaitre et al. (1987) Proc. Natl. Acad. Sci. USA 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) Bio-Techniques 6:958-976) or intercalating agents. (See, e.g., Zon (1988) Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

[00114] Another aspect of the invention pertains to isolated COX-1 variant proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-COX-1 variant antibodies. In one embodiment, native pCox-1 and COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

[00115] An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the COX-1 variant protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a COX-1 variant protein in which the protein is

separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of a COX-1 variant protein having less than about 30% (by dry weight) of non-protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non- protein, still more preferably less than about 10% of non- protein, and most preferably less than about 5% non- protein. When the COX-1 variant protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

[00116] The language "substantially free of chemical precursors or other chemicals" includes preparations of a protein of the invention in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of a COX-1 variant protein such as a COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) protein having less than about 30% (by dry weight) of chemical precursors or non- chemicals, more preferably less than about 20% chemical precursors or non- chemicals, still more preferably less than about 10% chemical precursors or non- chemicals, and most preferably less than about 5% chemical precursors or non- chemicals.

[00117] Biologically active portions of a COX-1 variant protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the COX-1 variant protein, e.g., the amino acid sequence shown in COX-1 variant, which include less amino acids than the full length proteins, and exhibit at least one activity of a protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the COX-1 variant protein. A biologically active portion of a protein can be a polypeptide which is, for example, at least 10, 25, 50, 100 or more amino acids in length.

[00118] In a preferred embodiment, the COX-1 variant protein has an amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO15 or SEQ ID NO:16. In other embodiments, the COX-1 variant protein is substantially homologous to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO15 or SEQ ID NO:16, and retains the functional activity of the protein of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO15 or SEQ ID NO:16, yet differs in amino acid sequence due to natural allelic variation or

mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the COX-1 variant protein is a protein which comprises an amino acid sequence at least about 50%, 55%, 59%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5 (e.g., the entire amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO15 or SEQ ID NO:16).

[00119] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[00120] The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6.

[00121] The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using

the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

[00122] The invention also provides COX-1 variant chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises a COX-1 variant polypeptide operatively linked to a non-COX-1 variant polypeptide. An "COX-1 variant polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a COX-1 variant, including COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs), whereas a "non-COX-1 variant polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to a protein of the invention, e.g., a protein which is different from a COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) protein and which is derived from the same or a different organism. Within a COX-1 variant fusion protein the COX-1 variant polypeptide can correspond to all or a portion of a protein. In a preferred embodiment, a COX-1 variant fusion protein comprises at least one biologically active portion of a COX-1 variant protein. In another preferred embodiment, a COX-1 variant fusion protein comprises at least two biologically active portions of a COX-1 variant protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the COX-1 variant polypeptide and the non- polypeptide are fused in-frame to each other. The non-polypeptide can be fused to the N-terminus or C-terminus of the COX-1 variant polypeptide.

[00123] For example, in one embodiment, the fusion protein is a GST- fusion protein in which the sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant COX-1 variants.

[00124] In another embodiment, the fusion protein is a COX-1 variant protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of a COX-1 variant can be increased through use of a heterologous signal sequence.

[00125] The fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject in vivo. The fusion proteins can be used to affect the bioavailability of a COX-1 variant substrate. Use of COX-1 variant fusion proteins may be useful therapeutically for the treatment of cellular growth related disorders or disorders associated with neurodegenerative disesaes. Moreover, the COX-1 variant -fusion proteins of the invention can be used as immunogens to produce anti- COX-1 variant antibodies in a subject, to purify ligands and in screening assays to identify molecules which inhibit the interaction of the enzyme with a substrate.

[00126] Preferably, a chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). An encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the protein.

[00127] The present invention also pertains to variants of the COX-1 variant proteins which function as either COX-1 variant agonists (mimetics) or as COX-1 variant antagonists. Variants of the COX-1 variant proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation of a COX-1 variant protein. An agonist of the proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a protein. An antagonist of a protein of the invention can inhibit one or more of the activities of the naturally occurring form of a protein of the invention by, for example, competitively modulating a cardiovascular system activity of a protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities

of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the protein.

[00128] A polypeptide "mutein" refers to a polypeptide whose sequence contains substitutions, insertions or deletions of one or more amino acids compared to the amino acid sequence of the native or wild type protein. A mutein has at least 50% sequence homology to the wild type protein, preferred is 60% sequence homology, more preferred is 70% sequence homology. Most preferred are muteins having 80%, 90% or 95% sequence homology to the wild type protein, in which sequence homology is measured by any common sequence analysis algorithm, such as Gap or Bestfit.

[00129] A "derivative" refers to polypeptides or fragments thereof that are substantially homologous in primary structural sequence but which include, e.g., in vivo or in vitro chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include but are not limited to, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzymatic modifications, or conservative substitutions, as will be readily appreciated by those well skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as ^{125}I, ^{32}P, ^{35}S, and ^{3}H, ligands which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods for labeling polypeptides are well known in the art. See Ausubel et al., 1992.

[00130] In one embodiment, a modified COX-1 variant protein which function as either a COX-1 variant agonists (mimetics) or as COX-1 variant antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a COX-1 variant protein (e.g., COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs)) agonist or antagonist activity. In one embodiment, a variegated library of modified COX-1 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of modified COX-1 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential COX-1 variant sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of COX-1 variant sequences therein.

There are a variety of methods which can be used to produce libraries of potential modified COX-1 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S.A. (1983) Tetrahedron 39:3; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al. (1984) Science 198:1056; Ike et al. (1983) Nucleic Acid Res. 11:477.

[00131] In addition, libraries of fragments of a COX-1 variants protein coding sequence can be used to generate a variegated population of COX-1 variants fragments for screening and subsequent selection of modified COX-1 variant protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a COX-1 variant coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the protein.

[00132] Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of COX-1 variant proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify modified COX-1 variants (Arkin and Yourvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815; Delgrave et al. (1993) Protein Engineering 6(3):327-331).

[00133] An isolated COX-1 variant protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind a COX-1 variant disclosed herein using standard techniques for polyclonal and monoclonal antibody preparation. A full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of, for example, COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) for use as immunogens. The antigenic peptide of a COX-1 variant comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO15 or SEQ ID NO:16, and encompasses an epitope of such that an antibody raised against the peptide forms a specific immune complex with . Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

[00134] Preferred epitopes encompassed by the antigenic peptide are regions of a COX-1 variant that are located on the surface of the protein, e.g., hydrophilic regions.

[00135] A COX-1 variant immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed protein or a chemically synthesized COX-1 variant polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic COX-1 variant preparation induces a polyclonal anti- antibody response.

[00136] Accordingly, another aspect of the invention pertains to anti- antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a COX-1 variant. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')2 fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind a COX-1 variant. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a COX-1 variant. A monoclonal antibody composition thus typically displays a single binding affinity for a particular COX-1 variant protein with which it immunoreacts.

[00137] Polyclonal anti- antibodies can be prepared as described above by immunizing a suitable subject with a COX-1 variant immunogen. The anti- antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized COX-1 variant. If desired, the antibody molecules directed against a COX-1 variant can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497) (see also, Brown et al. (1981) J. Immunol. 127:539-46; Brown et al. (1980) J. Biol. Chem .255:4980-83; Yeh et al. (1976) Proc. Natl. Acad. Sci. USA 76:2927-31; and Yeh et al. (1982) Int. J. Cancer 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) Immunol Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in Monoclonal Antibodies: A New Dimension In Biological Analyses, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) Yale J. Biol. Med., 54:387-402; M. L. Gefter et al. (1977) Somatic Cell Genet. 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds.

[00138] Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti- monoclonal antibody (see, e.g., G. Galfre et al. (1977) Nature 266:55052; Gefter et al. Somatic Cell Genet., cited supra; Lerner, Yale J. Biol. Med., cited supra; Kenneth, Monoclonal Antibodies, cited supra). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium").

Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind , e.g., using a standard ELISA assay.

[00139] Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti- antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with a COX-1 variant to thereby isolate immunoglobulin library members that bind a COX-1 variant. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. PCT International Publication No. WO 92/18619; Dower et al. PCT International Publication No. WO 91/17271; Winter et al. PCT International Publication WO 92/20791; Markland et al. PCT International Publication No. WO 92/15679; Breitling et al. PCT International Publication WO 93/01288; McCafferty et al. PCT International Publication No. WO 92/01047; Garrard et al. PCT International Publication No. WO 92/09690; Ladner et al. PCT International Publication No. WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum. Antibod. Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J 12:725-734; Hawkins et al. (1992) J. Mol. Biol. 226:889-896; Clarkson et al. (1991) Nature 352:624-628; Gram et al. (1992) Proc. Natl. Acad. Sci. USA 89:3576-3580; Garrad et al. (1991) Bio/Technology 9:1373-1377; Hoogenboom et al. (1991) Nuc. Acid Res. 19:4133-4137; Barbas et al. (1991) Proc. Natl. Acad. Sci. USA 88:7978-7982; and McCafferty et al. Nature (1990) 348:552-554.

[00140] Additionally, recombinant anti-COX-1 variant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by

recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent Application 173,494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al. European Patent Application 125,023; Better et al. (1988) Science 240:1041-1043; Liu et al. (1987) Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al. (1987) J. Immunol. 139:3521-3526; Sun et al. (1987) Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al. (1987) Canc. Res. 47:999-1005; Wood et al. (1985) Nature 314:446-449; and Shaw et al. (1988) J. Natl. Cancer Inst. 80:1553-1559); Morrison, S. L. (1985) Science 229:1202-1207; Oi et al. (1986) BioTechniques 4:214; Winter U.S. Patent 5,225,539; Jones et al. (1986) Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al. (1988) J. Immunol. 141:4053-4060.

[00141] An anti-COX-1 variant antibody (e.g., monoclonal antibody) can be used to isolate additional COX-1 variants, particularly those proteins retaining intron 1 of the COX-1 gene, by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-COX-1 variant antibody can facilitate the purification of natural COX-1 variant from cells and of recombinantly produced COX-1 variant expressed in host cells. Moreover, an anti- antibody can be used to detect a COX-1 variant protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the COX-1 variant protein. Anti-COX-1 variant antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical-testing procedure, e.g., ~~to, for example,~~ determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[00142] Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a COX-1 variant protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

[00143] The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be

transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., COX-1 variant proteins, modified forms of COX-1 variant proteins, fusion proteins, and the like).

[00144] The recombinant expression vectors of the invention can be designed for expression of COX-1 variant proteins in prokaryotic or eukaryotic cells. For example, COX-1 variant proteins can be expressed in bacterial cells such as E. coli, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

[00145] Expression of proteins in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

[00146] Purified fusion proteins can be utilized in COX-1 variant activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for COX-1 variant proteins, for example.

[00147] Examples of suitable inducible non-fusion E. coli expression vectors include pTrc (Amann et al., (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-

89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

[00148] One strategy to maximize recombinant protein expression in E. coli is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in E. coli (Wada et al., (1992) Nucleic Acids Res. 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

[00149] In another embodiment, the COX-1 variant expression vector is a yeast expression vector. Examples of vectors for expression in yeast S. cerevisiae include pYepSec1 (Baldari, et al., (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982) Cell 30:933-943), pJRY88 (Schultz et al., (1987) Gene 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

[00150] Alternatively, COX-1 variant proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

[00151] In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987) EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[00152] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) Genes Dev. 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) Adv. Immunol. 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) EMBO J. 8:729-733) and immunoglobulins (Banerji et al. (1983) Cell 33:729-740; Queen and Baltimore (1983) Cell 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) Science 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) Science 249:374-379) and the a-fetoprotein promoter (Campes and Tilghman (1989) Genes Dev. 3:537-546).

[00153] The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to COX-1 variant mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., "Antisense RNA as a molecular tool for genetic analysis," Reviews - Trends in Genetics, Vol. 1(1) 1986.

[00154] Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either

mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00155] A host cell can be any prokaryotic or eukaryotic cell. For example, a COX-1 variant protein can be expressed in bacterial cells such as E. coli, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[00156] Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

[00157] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a pCox-1 or pCox-1Δ657 protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

[00158] A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a COX-1 variant protein. Accordingly, the invention further provides methods for producing a COX-1 variant protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding COX-1 variant protein has been introduced) in a suitable medium such that a COX-1 variant protein is produced. In another embodiment, the method further comprises isolating a COX-1 variant protein from the medium or the host cell.

[00159] The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which -coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous COX-1 variant sequences have been introduced into their genome or homologous recombinant animals in which endogenous COX-1 variant sequences have been altered. Such animals are useful for studying the function and/or activity of a COX-1 variant such as COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs) and for identifying and/or evaluating modulators of their activity. A transgenic animal of the invention would include those animals that have been modified to express only a COX-1 variant protein (as opposed to co-expression of COX-1 and COX-1 variants). As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous COX-1 variant gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

[00160] The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: 1) screening assays; 2) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and 3) methods of treatment (e.g., therapeutic and prophylactic). The isolated nucleic acid molecules of the invention can be used, for example, to express COX-1 variant proteins (e.g., via a recombinant expression vector in a host cell), to detect COX-1 variant mRNA (e.g., in a biological sample), to modulate the activity of prostaglandins, and to identify compounds that modify the activity of a COX-1 variant. For example, the COX-1 variant proteins can be used to treat disorders characterized by insufficient or excessive production of a substrate or production of COX-1 variant inhibitors. In addition, the proteins of the invention can be used to screen for naturally occurring cyclooxygenase substrates, to screen for drugs or compounds which modulate the activity of a COX-1 variant, as well as to

treat disorders characterized by insufficient or excessive production of a COX-1 variant protein. Moreover, the anti-COX-1 variant antibodies of the invention can be used to detect and isolate COX-1 variant proteins, regulate the bioavailability of COX-1 variant proteins, and modulate COX-1 variant activity.

[00161] The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to COX-1 variant proteins (e.g., COX-3, PCOX-1a, PCOX-1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs)), have a stimulatory or inhibitory effect on, for example, COX-1 variant expression or COX-1 variant activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a COX-1 variant substrate. It is understood that the processes and methods for identifying (i.e., screening) compounds for COX-1 variant modulating activity include manufacturing any compound so identified. Accordingly, the invention contemplates a method for making a compound that modulates the activity of a COX-1 variant by providing a cell transfected with a DNA encoding a COX-1 variant, wherein the cell expresses the variant; contacting said cell, in an intact or disrupted state, with a test compound; and determining whether the activity of the COX-1 variant is decreased or increased in the presence of the test compound, wherein a decrease or increase in said Cox-3 activity is an indication that the test compound modulates the activity of Cox-3 and manufacturing the compound so identified.

[00162] In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of COX-1 variant protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a COX-1 variant protein or polypeptide or biologically active portion thereof, e.g., modulate the ability of COX-1 variant to interact with its cognate ligand. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) Anticancer Drug Des. 12:145). Combinatorial libraries are described in detail below.

[00163] In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a COX-1 variant target molecule (e.g., arachadonic acid) with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the COX-1 variant target molecule. Determining the ability of the test compound to modulate the activity of a COX-1 variant on a target molecule can be accomplished, for example, by determining the ability of the COX-1 variant protein to bind to or interact with the compound target molecule, or by determining the ability of the COX-1 variant protein to modify the test molecule.

[00164] Determining the ability of the COX-1 variant protein to bind to or interact with a target molecule can be accomplished by determining direct binding. Determining the ability of the COX-1 variant protein to bind to or interact with a COX-1 variant target molecule can be accomplished, for example, by coupling the COX-1 variant protein with a radioisotope or enzymatic label such that binding of the COX-1 variant protein to a COX-1 variant target molecule can be determined by detecting the labeled COX-1 variant protein in a complex. For example, COX-1 variant molecules, e.g., COX-1 variant proteins, can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, COX-1 variant molecules can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

[00165] It is also within the scope of this invention to determine the ability of a compound to modulate the interaction between COX-1 variant and its target molecule, without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of COX-1 variant with its target molecule without the labeling of a COX-1 variant or the target molecule. McConnell, H. M. et al. (1992) Science 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between compound and receptor.

[00166] In a preferred embodiment, determining the ability of the COX-1 variant protein to bind to or interact with a COX-1 variant target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic/enzymatic activity

of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., chloramphenicol acetyl transferase), or detecting a target-regulated cellular response.

[00167] One example of a simple in vitro system for the screening of compounds that modulate the activity of a COX-1 variant protein includes assays performed on living cells or on microsomal extracts prepared from the cultured cells. The COX-1 variant -synthesizing cell lines disclosed herein are useful for evaluating the activity of a compound on the activity of a COX-1 variant in comparison to the activity of the same compound on, for example, COX-1 or COX-2.

[00168] Thus, the present invention also provides a method to evaluate the relative inhibitory activity of a compound to selectively inhibit a COX-1 variant versus COX-1 or COX-2 activity, and thus to specifically inhibit the COX-1 variant activities associated with, for example, inflamed mammalian tissues, preferably human tissues, or in other physiological or pathological conditions in a mammalian host, preferably a human host. Such an assay can comprise contacting a COX-1 variant-expressing cell line or a microsomal extract thereof with a preselected amount of the compound in a suitable culture medium or buffer, adding a substrate (e.g., arachidonic acid) to the mixture, and measuring the level of synthesis of a COX-1 variant-mediated arachidonic acid metabolite, or the synthesis of any other metabolite unique to the cyclooxygenase pathway, by the cell line, or microsomal extract, as compared to a control cell line or portion of microsomal extract in the absence of said compound. The compound can be evaluated for its ability to selectively inhibit COX-1 variants or COX-1 or COX-2 by performing a second assay, in parallel, employing the above-described steps, with a COX-1 and/or COX-2 expressing cell line.

[00169] In yet another embodiment, an assay of the present invention is a cell-free assay in which a COX-1 variant protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the COX-1 variant protein or biologically active portion thereof is determined. Binding of the test compound to the COX-1 variant protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the COX-1 variant protein or biologically active portion thereof with a known compound which binds COX-1 variant to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a COX-1 variant protein, wherein determining the ability of the test compound to interact with a COX-1 variant protein

comprises determining the ability of the test compound to preferentially bind to or biologically active portion thereof as compared to the known compound.

[00170] For example, the present studies have identified COX-1 variants that are inhibited by compounds that have little or no inhibitory activity against COX-1 or COX-2, including analgesic/antipyretic compounds such as acetaminophen, phenacetin, antipyrine and dipyrone (see Table 1, and Figure 13). The screening methods described herein provide the means by which derivatives of such compounds can be identified for their selective inhibition of COX-1 variant activity. Such compounds are useful for treating a COX-1 variant-associated disorder in a subject. For example, acetaminophen is often categorized as a nonsteroidal antiinflammatory drug (NSAID) despite the fact that in clinical practice and in animal models it possesses little anti-inflammatory activity. Like NSAIDs, however, acetaminophen inhibits pain and fever. It has been shown that acetaminophen generally inhibits COX activity in dog brain homogenates more than in homogenates from spleen (Flower and Vane, (1972) Nature 240:410-411). However, as previous and current studies indicate, neither COX-1 nor COX-2 is inhibited by acetaminophen at physiological concentrations of the drug in whole cells or homogenates (Botting (2000) Clin. Infect. Dis. 31:8202-8210.) suggesting that neither isozyme is a good candidate for the site of action of acetaminophen. The present study indicates that COX-1 variants are clearly enriched in dog brain and the human 5.2kb transcript is highest in brain cortex. Further, epidemiologic evidence indicates that NSAID use is associated with a lower incidence or risk of Alzheimer's Disease (AD). An inverse relationship is seen between NSAID use (particularly aspirin), and AD incidence in case-controlled studies of patients who have osteoarthritis, rheumatoid arthritis, or who use NSAIDs for other purposes. A similar inverse correlation was seen in a co-twin control study of 50 elderly twins with AD onset separated by 3 years or more. Both decreased risk of AD among NSAID users as well as an decreased risk of AD with increased duration of NSAID use was found in the prospective Baltimore Longitudinal Study of Aging and a decrease in cognitive decline was associated with NSAID use in the 1-year Rotterdam Study.

[00171] In another embodiment, the assay is a cell-free assay in which a COX-1 variant protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the COX-1 variant protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a COX-1 variant protein can be accomplished, for example, by determining the ability of the COX-1 variant protein to bind to a COX-1 variant target molecule by one of the methods described above for determining direct binding.

Determining the ability of the COX-1 variant protein to bind to a COX-1 variant target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) Anal. Chem. 63:2338-2345 and Szabo et al. (1995) Curr. Opin. Struct. Biol. 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

[00172] In yet another embodiment, the cell-free assay involves contacting a COX-1 variant protein or biologically active portion thereof with a known compound which binds the COX-1 variant protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the COX-1 variant protein, wherein determining the ability of the test compound to interact with the COX-1 variant protein comprises determining the ability of the protein to preferentially bind to or modulate the activity of a target molecule.

[00173] In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either COX-1 variant or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a COX-1 variant protein, or interaction of a COX-1 variant protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ COX-1 variant fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or COX-1 variant protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the

matrix, and the level of COX-1 variant binding or activity determined using standard techniques.

[00174] In another embodiment, modulators of COX-1 variant expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of COX-1 variant mRNA or protein in the cell is determined. The level of expression of COX-1 variant mRNA or protein in the presence of the candidate compound is compared to the level of expression of COX-1 variant mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of COX-1 variant expression based on this comparison. For example, when expression of COX-1 variant mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of COX-1 variant mRNA or protein expression. Alternatively, when expression of COX-1 variant mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of COX-1 variant mRNA or protein expression. The level of COX-1 variant mRNA or protein expression in the cells can be determined by methods described herein for detecting COX-1 variant mRNA or protein.

[00175] In another embodiment, the nucleic acid and polypeptide sequences disclosed herein provide a method for making structure-based predictions about the behavior of a COX-1 variant enzyme in the presence or absence of a test compound. Such in silico methods are based on mathematical algorithms that manipulate various types of structural information based in part on the primary amino acid structure of a polypeptide. Additional information generated from homologous or partially homologous proteins can be integrated into the method in order to augment the amino acid structure information. Thus, the polypeptide sequence information disclosed herein for novel COX-1 variant proteins can be used, in conjunction with structural information available from COX-1 and COX-2 studies, to predict which compounds, or family of compounds, will specifically interact with a COX-1 variant protein. The entire process can be accomplished in silico by algorithms known to those skilled in the art.

[00176] For example, substantial information regarding COX structural motifs and their effect on functional activity is available. COX-1 and COX-2 dimers are held together via molecular interactions involving the dimerization domains of each monomer. Heterodimerization of COX-1 and COX-2 subunits does not occur. The dimerization domain is encoded by approximately 50 amino acids near the amino terminus of the proteolytically

processsed protein. Three disulfide bonds hold this domain together in a structure reminiscent of epidermal growth factor (EGF). A fourth disulfide bond links the dimerization domain with the globular catalytic domain. The presence of disulfide bonds, which require an oxidizing environment to form, is consistent with the concept that COX-1 and COX-2 are located inside the lumen of the nuclear envelope, ER, or golgi, which have redox states that are significantly less reduced than cytosol.

[00177] COX isozymes associate with the intralumenal surface of microsomal membranes in an unusual fashion. Rather than employing transmembrane spanning sequences or covalently-bound lipids for attachment, COX isozymes contain a tandem series of four amphipathic helices which creates a hydrophobic surface that penetrates into the lumenal-side of the hydrophobic core of the lipid bilayer. These helices are encoded by approximately 50 amino acids found immediately carboxyterminal to the bulk of the dimerization domain. The helices allow COX dimers to attach to the inside surface of the lumen of the ER/nuclear envelope, with the majority of the protein protruding into the lumenal space of these compartments. The membrane binding domain also forms the mouth of a narrow, hydrophobic channel that is the cyclooxygenase active site.

[00178] Carboxy-terminal to the membrane binding domain in COX primary structures is the catalytic domain, which comprises 80% (approximately 480 amino acids) of the protein and contains two distinct enzymatic active sites. The first is a peroxidase (POX) active site. The entire catalytic domain of COX isozymes is globular with 2 distinct intertwining lobes. The interface of these lobes creates a shallow cleft on the upper surface of the enzyme (i.e. the surface furthest from the membrane) where the peroxidase active site is located and where heme is bound. Coordination of the heme is via an iron-histidine bond involving His 388 in sheep COX-1. Other important interactions between the protoporphyrin also occur and specific amino acids which may function in coordinating PGG2 have been identified. The geometry of heme binding leaves a large portion of one side of the heme exposed in the open cleft of the peroxidase active site for interaction with PGG2 and other lipid peroxides.

[00179] The second distinct enzymatic active site in the catalytic domain is a cyclooxygenase (COX) active site. The cyclooxgyenase active site is a long, narrow, dead-end channel of largely hydrophobic character whose entrance is framed by the four amphipathic helices of the membrane binding domain. The channel extends approximately 25 angstroms into the globular catalytic domain and is on average about 8 angstroms wide. However, significant narrowing of the channel is observed where arginine 120, one of only two ionic residues found in the COX active site, protrudes into the channel and forms a

hydrogen bonded network with glutamate 524 (the other ionic residue in the channel) and tyrosine 355. Arginine 120 is essential for substrate binding in COX-1 but appears to be significantly less important in COX-2. Arginine 120 is also clearly important in the binding of carboxylate-containing NSAIDs in the COX-1 active site but as with AA binding, it is significantly less important in coordinating these NSAIDs in COX-2.

[00180] The upper portion of the channel, or catalytic pocket, contains tyrosine 385 that forms a tyrosyl radical, abstracts hydrogen from the pro-S side of carbon 13 of AA, and creates an activated fatty acid radical that undergoes the cyclization and/or oxygenation reaction (see Figure 3). Also in the hydrophobic pocket is Ser 530, which is transacetylated by aspirin. The hydroxyl of serine 530 itself is not essential for catalysis. However, its acetylation prevents abstraction of hydrogen from AA in COX-1 by sterically preventing AA from binding productively in the active site. In contrast, abstraction of hydrogen does occur in acetylated COX-2, but cyclization of the fatty acid radical and formation of the endoperoxide does not occur, yielding 15-R-hydroxyeicosotetraenoic acid (15R-HETE) rather than COX-2.

[00181] A structural difference between the active sites of COX-1 and COX-2 is a substitution of isoleucine 523 in COX-1 for a valine in COX-2. This single difference opens a hydrophobic outpocketing in COX-2 that can be accessed by some COX-2 selective drugs. There are other changes in residues that are near but do not line the COX active site, so-called second shell residues, that result in subtle changes and a slightly enlarged COX-2 active site relative to COX-1.

[00182] As previously noted, NSAIDs are analgesic/antiinflammatory/antipyretic medications that act as inhibitors of the cyclooxygenase active site of COX isozymes. Important mechanistic differences in the actions of individual NSAIDs with the COX active site exist. Of the NSAIDs in medical use, only aspirin is a covalent modifier of COX-1 and COX-2. The crystallographic studies of Garavito and colleagues demonstrated why this drug so efficiently acetylates serine 530 of COX-1 (Loll,1995, cite). Like other NSAIDs, aspirin diffuses into the COX active site of enzyme through the mouth of the channel and traverses up the channel to the constriction point formed by Arg 120, Tyr 355, and Glu 524. At this point in the channel, the carboxyl of aspirin forms a weak ionic bond with the side-chain of Arg 120. This positions aspirin only 5 angstroms below Ser 530 and in the correct orientation for transacetylation. Because the catalytic pocket of the channel is somewhat larger in COX-2 than in COX-1, orientation of aspirin for attack on Ser 530 is not as good in

COX-2 and transacetylation efficiency is reduced. This accounts for the 10-100-fold lowered sensitivity to aspirin of COX-2 in comparison to COX-1.

[00183] Other NSAIDs in addition to aspirin inhibit COX-1 and COX-2 by competing with AA for binding in the COX active site. However, NSAIDs significantly differ from each other in whether they bind the COX active site in a time-dependent or independent fashion. For example, NSAIDs differ dramatically with regard to how quickly they productively bind in the COX active site and how quickly they come out of the COX channel. Some NSAIDs, such as ibuprofen, have very rapid on and off rates. They inhibit COX activity essentially instantaneously after addition of the NSAID and they readily wash out of the COX active site when the NSAID is removed from the environment of the enzyme. In contrast, many NSAIDs such as indomethacin and diclofenac are time-dependent. They require typically seconds to minutes to bind the COX active site. Once bound, however, these drugs typically have low off-rates that may require many hours for the NSAID to wash out of the active site. Time-dependent NSAIDs compete very poorly with AA in instantaneous assays of COX activity. Co-crystallization studies have been performed for flurbiprofen and COX-1 and COX-2 as well as indomethacin and COX-1 which define the precise binding interactions of carboxyl-containing NSAIDs in the COX binding site.

[00184] NS398 is a particularly important COX-2 inhibitor which is commercially available and, therefore, is widely used in pharmacology studies. Celecoxib, rofecoxib, and NS398 have been co-crystallized with COX-2. Celecoxib and rofecoxib are diaryl compounds containing a sulfonamide and methylsulfone, respectively, rather than a carboxyl group. Hence, the identification of COX isozymes has allowed the eventual synthesis and testing of NSAIDs, in the form of celecoxib, rofecoxib, that have resulted in important therapeutic agents.

[00185] By combining the structural information available for COX-1 and COX-2 with the novel sequence information for COX-1 variants disclosed herein and the compound inhibition studies disclosed herein, one skilled in the art can predict the three-dimensional structure of a COX-1 variant and subsequently select potential inhibitors of a COX-1 variant. Accordingly, the invention encompasses a method for identifying a potential inhibitor for a COX-1 variant by providing a three-dimensional structure of the COX-1 variant as defined by atomic coordinates and employing the three-dimensional structure to design or select a potential inhibitor. The method further involves synthesizing the potential inhibitor and contacting the potential inhibitor with the COX-1 variant in the presence or absence of a

substrate of COX-1 variant to determine the ability of the potential inhibitor to inhibit enzyme Z.

[00186] The invention further provides a method for identifying a potential inhibitor of a COX-1 variant by providing the three-dimensional coordinates of an inhibitor when it is bound to COX-1 variant and comparing the three-dimensional coordinates of the inhibitor when it is bound to the COX-1 variant to the three-dimensional coordinates of compounds in a database of compound structures and selecting from the database at least one compound that is structurally similar to the inhibitor when it is bound to the COX-1 variant.

[00187] The invention further provides a method for identifying a potential inhibitor of a COX-1 variant by providing the three-dimensional coordinates and identities of the atoms of inhibitor I when it is bound to the COX-1 variant; selecting a subset of atoms of the inhibitor that govern its interaction with the COX-1 variant; comparing the three-dimensional coordinates of the selected subset of atoms of the inhibitor with the three-dimensional coordinates of compounds in a database of compound structures; and selecting from the database at least one compound comprising three-dimensional coordinates that are identical to the three-dimensional coordinates of the selected subset of atoms of inhibitor, wherein the selected compound is a potential inhibitor of a COX-1 variant.

[00188] In another embodiment, the novel polypeptides of the invention can be expressed and crystallized in the presence of absence of a test compound. Once crystallized, the three-dimensional structure of a COX-1 variant polypeptide may be determined in a number of ways. Many of the most precise methods employ X-ray crystallography (for a general review, see, Van Holde, Physical Biochemistry, Prentice-Hall, N. J. pp. 221-239, (1971), which is incorporated herein by reference). This technique relies on the ability of crystalline lattices to diffract X-rays or other forms of radiation. Diffraction experiments suitable for determining the three-dimensional structure of macromolecules typically require high-quality crystals. Various methods for preparing crystalline proteins and polypeptides are known in the art (see, for example, McPherson, et al. "Preparation and Analysis of Protein Crystals", A. McPherson, Robert E. Krieger Publishing Company, Malabar, Fla. (1989); Weber, Advances in Protein Chemistry 41:1-36 (1991); U.S. Pat. No. 4,672,108; and U.S. Pat. No. 4,833,233; all of which are incorporated herein by reference for all purposes).

[00189] This invention further pertains to novel agents or compounds identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an compound identified as described herein in an appropriate animal model. For example, an compound identified as described herein (e.g., a COX-1 variant modulating

compound, an antisense COX-1 variant nucleic acid molecule, a COX-1 variant -specific antibody, or a COX-1 variant -binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an compound. Alternatively, an compound identified as described herein can be used in an animal model to determine the mechanism of action of such an compound. Furthermore, this invention pertains to uses of novel compounds identified by the above-described screening assays for treatments as described herein.

[00190] The invention further contemplates the use of high-throughput screening techniques to identify candidate compounds that modulate the activity of COX-1 variants of the invention. Conventionally, new chemical entities with useful properties are generated by identifying a chemical compound (called a "lead compound") with some desirable property or activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. However, the current trend is to shorten the time scale for all aspects of drug discovery. Because of the ability to test large numbers quickly and efficiently, high throughput screening (HTS) methods are replacing conventional lead compound identification methods.

[00191] In one preferred embodiment, high throughput screening methods involve providing a library containing a large number of potential therapeutic compounds (candidate compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

[00192] Combinatorial chemical libraries are a preferred means to assist in the generation of new chemical compound leads. A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks called amino acids in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks. For example, one commentator has observed that the systematic, combinatorial mixing of 100 interchangeable chemical building blocks results in the theoretical synthesis of

100 million tetrameric compounds or 10 billion pentameric compounds (Gallop et al. (1994) 37(9): 1233-1250).

[00193] Preparation and screening of combinatorial chemical libraries are well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka (1991) Int. J. Pept. Prot. Res., 37: 487-493, Houghton et al. (1991) Nature, 354: 84-88). Peptide synthesis is by no means the only approach envisioned and intended for use with the present invention. Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (PCT Publication No WO 91/19735, 26 Dec. 1991), encoded peptides (PCT Publication WO 93/20242, 14 Oct. 1993), random bio-oligomers (PCT Publication WO 92/00091, 9 Jan. 1992), benzodiazepines (U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., (1993) Proc. Nat. Acad. Sci. USA 90: 6909-6913), vinylogous polypeptides (Hagihara et al. (1992) J. Amer. Chem. Soc. 114: 6568), nonpeptidal peptidomimetics with a Beta-D-Glucose scaffolding (Hirschmann et al., (1992) J. Amer. Chem. Soc. 114: 9217-9218), analogous organic syntheses of small compound libraries (Chen et al. (1994) J. Amer. Chem. Soc. 116: 2661), oligocarbamates (Cho, et al., (1993) Science 261:1303), and/or peptidyl phosphonates (Campbell et al., (1994) J. Org. Chem. 59: 658). See, generally, Gordon et al., (1994) J. Med. Chem. 37:1385, nucleic acid libraries, peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083) antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3): 309-314), and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. (1996) Science, 274: 1520-1522, and U.S. Patent 5,593,853), and small organic molecule libraries (see, e.g., benzodiazepines, Baum (1993) C&EN, Jan 18, page 33, isoprenoids U.S. Patent 5,569,588, thiazolidinones and metathiazanones U.S. Patent 5,549,974, pyrrolidines U.S. Patents 5,525,735 and 5,519,134, morpholino compounds U.S. Patent 5,506,337, benzodiazepines 5,288,514, and the like).

[00194] Devices for the preparation of combinatorial libraries are commercially available (see, e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). A number of well known robotic systems have also been developed for solution phase chemistries. These systems include automated workstations like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, Hewlett-Packard, Palo Alto, Calif.) which mimic the manual synthetic operations performed

by a chemist. Any of the above devices are suitable for use with the present invention. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art. In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

[00195] Any of the assays for compounds capable of modulating COX-1 variant activity described herein are amenable to high throughput screening. High throughput screening systems are commercially available (see, e.g., Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like.

[00196] Portions or fragments of the novel cDNA sequences identified herein can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample.

[00197] Monitoring the influence of agents (e.g., drugs or compounds) on the expression or activity of a COX-1 variant protein can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase COX-1 variant gene expression, protein levels, or upregulate COX-1 variant activity, can be monitored in clinical trials of subjects exhibiting decreased COX-1 variant transcript expression, protein levels, or downregulated COX-1 variant activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease COX-1 variant gene expression, protein levels, or downregulate COX-1 variant activity, can be monitored in clinical trials of subjects exhibiting increased COX-1 variant gene expression, protein levels, or upregulated COX-1 variant activity. In such clinical trials,

the expression or activity of a gene, and preferably, other genes that have been implicated in a disorder can be used as a "read out" or markers of the phenotype of a particular cell.

[00198] In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a COX-1 variant protein, mRNA in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the protein or mRNA in the post-administration samples; (v) comparing the level of expression or activity of the protein or mRNA in the pre-administration sample with the protein or mRNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of a COX-1 variant to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of COX-1 variant to lower levels than detected, i.e. to decrease the effectiveness of the agent. According to such an embodiment, COX-1 variant expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

[00199] The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant COX-1 variant expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the COX-1 variant molecules of the present invention or modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic

treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

[00200] Accordingly, the present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a COX-1 variant protein; (ii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a COX-1 variant protein, wherein a wild-type form of the gene encodes a protein with a COX-1 variant activity. The invention further provides diagnostic assays for determining the relative expression levels of a COX-1 variant transcript or polypeptide in relation to a COX-1 or COX-2 transcript or polypeptide. A diagnostic assay can include, for example, an array-based system for detecting the presence or absence of a COX-1 variant or the presence or absence of a genetic alteration in a COX-1 variant. An array-based system can include a) bead arrays, bead based arrays, bioarrays, bioelectronic arrays, cDNA arrays, cell arrays, DNA arrays, encoded bead arrays, gel pad arrays, gene arrays, gene expression arrays, genome arrays, genomic arrays, high density oligonucleotide arrays, high density protein arrays, hybridization arrays, in situ arrays, low density arrays, microelectronic arrays, multiplex DNA hybridization arrays, nanoarrays, nylon macroarrays, oligo arrays, oligonucleotide arrays, oligosaccharide arrays, peptide arrays, planar arrays, protein arrays, solution arrays, spotted arrays, tissue arrays, exon arrays, filter arrays, macroarrays, small molecule microarrays, suspension arrays, theme arrays, tiling arrays or transcript arrays that incorporate.

[00201] For example, an array comprising a nucleic acid, protein or polypeptide of the invention or molecule that interacts with a nucleic acid, protein or polypeptide of the invention can be used in detection assays, diagnostic assays and in assays for monitoring the effects of a compound during clinical trials. Accordingly, such an array can include a nucleic acid, protein or polypeptide of the invention or molecule that interacts with a nucleic acid, protein or polypeptide disclosed herein, including COX-3, PCOX-1a, PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs).

[00202] In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant COX-1 variant expression or activity, by administering to the subject a COX-1 variant or a compound which modulates COX-1 variant expression or at least one COX-1 variant activity. Subjects at risk for a disease which is caused or contributed to by aberrant COX-1 variant expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms

characteristic of the COX-1 variant aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of COX-1 variant aberrancy, for example, a COX-1 variant, agonist or antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

[00203] Accordingly, the invention provides a method of selecting a therapy for a patient by providing a subject expression profile of a sample from said patient; providing a plurality of reference profiles, each associated with a therapy, wherein the subject expression profile and each reference profile has a plurality of values, each value representing the expression level of a COX-1 variant transcript or polypeptide; and selecting the reference profile most similar to the subject expression profile, to thereby select a therapy for said patient.

[00204] The invention further provides an array comprising a substrate having a plurality of addresses, wherein each address has disposed thereon a capture probe that can specifically bind a COX-1 variant nucleic acid. The nucleic acids can be selected from the group consisting of COX-1 variant SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13. The substrate can possess a range of addresses, each corresponding to a unique COX-1 variant sequence. The range of addresses can include 5 – 10,000, depending on the density of the array.

[00205] The invention further provides a method of choosing a therapy for a patient, by providing a plurality of reference expression profiles, each associated with a therapy; providing a nucleic acid obtained from a patient; contacting the nucleic acid with the array comprising a substrate having a plurality of addresses, wherein each address has disposed thereon a capture probe that can specifically bind a COX-1 variant nucleic acid; detecting binding of the nucleic acid to each address of the plurality of addresses to thereby provide a subject expression profile; and selecting the reference profile most similar to the subject expression profile, to thereby choose a therapy for said patient.

[00206] The invention further provides a method for evaluating whether or not a pharmaceutical composition will be effective for interacting with a COX-1 variant. The method can utilize an array having a substrate including a plurality of addresses, wherein each address has disposed thereon a capture probe that can specifically bind a COX-1 variant nucleic acid; and a computer-readable medium having a plurality of digitally-encoded expression profiles wherein each profile of the plurality has a plurality of values, each value representing the expression of a COX-1 variant nucleic acid detected by the array.

[00207] The invention further encompasses a method of selecting a therapy for a subject by obtaining a subject sample from a caregiver; obtaining a nucleic acid from the subject sample; identifying a subject expression profile from the nucleic acid; selecting from a plurality of reference profiles a matching reference profile most similar to the subject expression profile, wherein the reference profiles and the subject expression profile have a plurality of values, each value representing the expression level of a COX-1 variant, wherein each reference profile of the plurality of reference profiles is associated with a therapy; and transmitting a descriptor of the therapy associated with the matching reference profile to the caregiver, thereby selecting a therapy for said subject.

[00208] The invention further provides a kit for evaluating a pharmaceutical composition, the kit comprising an array as described above and a computer-readable medium having a plurality of expression profiles, wherein each profile of the plurality has a plurality of values, each value representing the expression of a COX-1 variant nucleic acid detected by the array.

[00209] Another aspect of the invention pertains to methods of modulating COX-1 variant expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a COX-1 variant or agent that modulates one or more of the activities of COX-1 variant protein activity associated with the cell. A compound or agent that modulates COX-1 variant protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a COX-1 variant protein, a COX-1 variant antibody, a COX-1 variant agonist or antagonist, a peptidomimetic of a COX-1 variant agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more COX-1 variant activities. Examples of such stimulatory agents include active COX-1 variant protein and a nucleic acid molecule encoding COX-1 variant that has been introduced into the cell. In another embodiment, the agent inhibits one or more COX-1 variant activites. Examples of such inhibitory compounds or agents include antisense COX-1 variant nucleic acid molecules, anti-COX-1 variant antibodies, and COX-1 variant inhibitors. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo (e.g, by administering the agent to a subject) or even in silico, as described elsewhere. As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of COX-1 variant protein or nucleic acid molecule.

[00210] The COX-1 variant nucleic acid molecules, COX-1 variant proteins, compounds identified as modulating a COX-1 variant activity and anti- COX-1 variant antibodies (also

referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, compound or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00211] For example, epidemiologic and clinical data suggest that the use non-steroidal anti-inflammatory drugs (NSAIDs) delays the onset of AD and reduces the progression of pathologic symptoms in Alzheimer's disease. McGeer and McGeer, Brain Res. Rev. 21, 195 (1995). Aspirin, like most NSAIDs, prevent inflammation and pain by inhibiting both COX-1 and COX-2 enzymes. Resveratrol, a phenolic antioxidant and COX inhibitor found in grapes, inhibits prostaglandin production, and has anti-cancer and anti-inflammatory properties. Jang et al., Science 275, 218 (1997). Alzheimer's Disease (AD) is the most common neurodegenerative disorder of aging, and is characterized by progressive dementia and personality dysfunction. The abnormal accumulation of amyloid plaques in the vicinity of degenerating neurons and reactive astrocytes is a pathological characteristic of AD. The present invention further relates to compositions and methods for the treatment of various neurological diseases and neurodegenerative disorders. For example, a composition that modulates a COX-1 variant of the invention can be used to treat neurodegenerative diseases associated with an overabundance of Amyloid Precursor Protein (APP). Cytosolic phospholipase A2, which releases arachidonic acid from cellular phospholipids, is elevated in AD brain and after transient global ischemia. The cyclooxygenation of arachidonic acid, catalyzed by two forms of cyclooxygenase (COX), COX-1 and COX-2, produces prostaglandins which, in turn, regulate neurotransmission, immune and inflammatory responses by activating receptors coupled to cAMP formation. cAMP elevations caused by activation of neurotransmitter receptors increased APP mRNA and holoprotein production in astrocytes.

[00212] Accordingly, active compounds identified by a screening assay disclosed herein can be in included in a pharmaceutical composition in order to ameliorate a COX-1 variant associated disorder such as, for example, a neurodegenerative condition or disease. The

condition can be treated in a subject by administering a specific inhibitor of COX-1 variant activity as disclosed in the invention, in a pharmaceutically acceptable carrier. It is further object of the invention to provide a method for preventing or treating Alzheimer's Disease in a subject by administering an effective amount of a specific inhibitor of COX-1 variant activity as disclosed in the present invention.

[00213] The invention further provides a method for treating immune or inflammatory conditions associated with a neurodegenerative condition in a subject by administering a specific inhibitor of a COX-1 variant as provided in the this disclosure. The invention encompasses a method of modulating expression, production, or formation of amyloid precursor protein (APP) in a subject by administering an effective amount of an antagonist of a COX-1 variant of the invention. The antagonist can be, for example, a non-steroidal anti-inflammatory agent that is a specific inhibitor of COX-1 variant activity, in a pharmaceutically acceptable carrier. Thus, the present invention provides methods and compositions that can modulate or regulate the production or formation of APP in patients, including the expression of APP gene products and the transcription or translation of the APP gene in central nervous system. For example, the production of APP by mammalian cells, in particular, by cells in the brain, can be increased or reduced. In attaining this objective, it is also an objective of the invention to inhibit excessive amyloid formation, prevent neurite dystrophy and alleviate pathological symptoms, such as neurodegeneration or cognitive deficits that may arise from the negative effects of inappropriately expressed, produced, or formed amounts of APP.

[00214] Accordingly, the present invention is useful in the treatment or alleviation of disease, especially those disorders related to neurological diseases or neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Lou Gehrig's disease, or multiple sclerosis, to name a few, not to mention central or peripheral nervous system damage, dysfunction, or complications involving same stemming from edema, injury, or trauma. Such damage, dysfunction, or complications may be characterized by an apparent neurological, neurodegenerative, physiological, psychological, or behavioral aberrations, the symptoms of which can be reduced by the administration of an effective amount of the active compounds or substances of the present invention.

[00215] According to one embodiment, the administration of effective amounts of a composition that modulates the activity of a COX-1 variant of the invention can be used to suppress, inhibit, or neutralize the action of increased cAMP activity, which activity if unchecked leads to the overproduction of APP. A variety of non-steroidal anti-inflammatory

agents (NSAIDs) are found to be suitable for reversing the stimulatory effects of cAMP, its derivatives, a ligand, an agonist, or an antagonist of a receptor that is coupled to the cellular levels of cAMP, or a compound that enhances the nuclear actions of cAMP. Examples of suitable NSAIDs include, but are not limited to, Advil, Aspirin, Aleve, Anaprox, Diclofenac, Docosahexaenoic acid, Dolobid, Etodolac, Feldene, Flurbiprofen, Ibuprofen, Indomethacin, Ketorolac tromethamine, Lodine, Meclofenamate, 6-MNA, Motrin, Nalfon, Naprosyn, Nuprin, Orudis, Phenylbutazone, Piroxicam, Phenylbutazone, Ponstel, Relafen, Salicylic acid, Sulindac sulfide, Tolectin, Toradol, Voltaren; also 5-lipoxygenase inhibitors, phosphodiesterase inhibitors, or cyclooxygenase inhibitors (e.g., cyclosalicylazosulfapyridine, azulfasalazine, DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(5H)-furanone), or DFP (5,5-dimethyl-3-isopropyloxy-4-(4'-methylsulfonylphenyl)-2(5H)-furanone).

[00216] As used herein, the term "central nervous system" refers to all structures within the dura mater. Such structures include, but are not limited to, the brain and spinal cord.

[00217] As used herein, the terms "subject suffering from Alzheimer's disease," "subject suffering from a disease with an inflammatory component," and "subject suffering from central nervous system injury," refer to subjects that are identified as having or likely having the particular disease, injury, or condition, respectively. As used herein the terms "subject susceptible to Alzheimer's disease" and "subject susceptible to a disease with an inflammatory component," refer to subjects identified as having a risk of contracting or developing the particular disease, injury, or condition, respectively. As used herein, the term "disease with an inflammatory component" refers to diseases and conditions that are associated with an inflammatory element. The inflammatory element can comprise a symptom, side-effect, or causative event associated with the disease or condition. Diseases with an inflammatory component include, but are not limited to, stroke, ischemic damage to the nervous system, neural trauma (e.g., percussive brain damage, spinal cord injury, and traumatic damage to the nervous system), multiple sclerosis and other immune-mediated neuropathies (e.g., Guillain-Barre syndrome and its variants, acute motor axonal neuropathy, acute inflammatory demyelinating polyneuropathy, and Fisher Syndrome), HIV/AIDs dementia complex, and bacterial and viral meningitis. Such diseases further include degenerative diseases, such as Alzheimer's Disease (AD), Parkinson's Disease (PD), Amyotrophic lateral sclerosis (ALS), Huntington's Disease (HD), Pick's disease, progressive supranuclear palsy, striatonigral degeneration, cortico-basal degeneration, olivopontocerebellar atrophy, Leigh's disease, infantile necrotizing encephalomyelopathy,

Hunter's disease, mucopolysaccharidosis, various leukodystrophies (such as Krabbe's disease, Pelizaeus-Merzbacher disease and the like), amaurotic (familial) idiocy, Kuf's disease, Spielmayer-Vogt disease, Tay Sachs disease, Batten disease, Jansky- Bielschowsky disease, Reye's disease, cerebral ataxia, chronic alcoholism, beriberi, Hallervorden-Spatz; syndrome, cerebellar degeneration, and the like.

[00218] As used herein, the term "neurological defect" refers to a defect involving or relating to the nervous system. Some neurological defects are caused by defective tissues or cells of the nervous system, while other defects are caused by defective tissues or cells that affect the nervous system. As used herein, the term "neurologically defective mammal" refers to a mammal having one or more neurological defects. When a neurological defect is "ameliorated," the condition of the host is improved. For example, amelioration can occur when defective tissue is returned partially or entirely to a normal state. However, amelioration can also occur when tissue remains subnormal, but is otherwise altered to benefit the host. As used herein, the term "lesion" refers to a wound or injury, or to a pathologic change in a tissue.

[00219] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00220] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under

the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00221] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00222] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as

colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00223] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[00224] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00225] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00226] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00227] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic

effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[00228] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00229] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00230] The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[00231] The invention also encompasses pharmaceutical compositions comprising a compound identified by a method of the invention contained in a container and labeled with instructions for use as a COX-1variant specific inhibitor. The pharmaceutical composition

con be included in a kit with instructions for use of the composition in the treatment of a COX-1 variant associated disorder. The kit can further comprise instructions for using dosage. Accordingly, the invention contemplates an article of manufacture comprising packaging material and, contained within the packaging material, a compound that modulates the activity of a COX-1 variant, such as, for example, Cox-3, wherein the packaging material comprises a label or package insert indicating that said compound modulates the activity of a COX-1 variant and can be used for treating (pain/inflammation) in a subject. The invention further contemplates an article of manufacture comprising packaging material and, contained within the packaging material, a compound that preferentially modulates the activity of a COX-1 variant in comparison to Cox-1 and Cox-2, wherein the packaging material comprises a label or package insert indicating that said compound modulates the activity of a COX-1 variant and can be used for treating (pain/inflammation) in a subject.

Examples

[00232] Recent studies have demonstrated that COX-1 possesses at least three distinct domains responsible for dimerization, membrane binding, and catalysis. A fourth domain, the N-terminal signal peptide, which is clearly evident in the primary structure of COX-1, was not observed because this sequence typically is co-translationally cleaved from the nascent polypeptide by microsomal signal peptidase. The amino terminal signal peptide directs synthesis of the COX isozymes into the lumen of the endoplasmic reticulum/nuclear envelope. Although cleaved from the nascent polypeptide, the amino terminal hydrophobic signal peptide shows a size difference between COX-1 and COX-2 that, prior to the present invention, had unknown biological significance. The signal peptide for COX-1 is generally 22-29 amino acids in length with a large hydrophobic core comprised of 4 or more leucines or isoleucines. This sequence is encoded by exons 1 and 2 of the COX-1 gene. Exon 2 of COX-1 terminates precisely at the cleavage site for the signal peptide. In contrast to COX-1, COX-2's signal peptide is 17 amino acids long in all species and it is encoded entirely by exon 1 of the COX-2 gene, which terminates precisely at the cleavage site for the signal peptide. Therefore, the hydrophobic signal peptide is precisely encoded by exons 1 and 2 in COX-1 and exon 1, alone, in the COX-2 gene. Intron 1 of the COX-1 gene is missing in the COX-2 gene. This represents a major difference in structure between the COX-1 and COX-2 genes.

[00233] In vitro translation experiments have demonstrated that COX-1 is rapidly translocated into the lumen of canine pancreatic microsomes, whereas COX-2 is inefficiently

translocated (Xie, et al. (1991) Proc. Natl. Acad. Sci. USA 88:2692-2696.). To date this is the only known biochemical property of the COX-1 and COX-2 isozymes to be affected by this difference in the length of the signal peptide. However, the discovery described herein shows that regulation of this important signal sequence by intron retention in COX-1 occurs in brain and other tissues to create unique variants (i.e., COX-1 variants) of COX isozymes.

[00234] Accordingly, novel mRNAs encoding COX-variant proteins (e.g., COX-3, PCOX-1a, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs)) are expressed in mammalian tissue and are provided herein. For example, canine and human cerebral cortex express COX-1 variant mRNA. In addition, human COX-3 mRNA (~5.2 kb transcript) has been identified from heart tissue. Intron 1 is conserved in length and in sequence in mammalian COX-1 genes. This intron contains an open reading frame that introduces an insertion of 30-34 amino acids, depending on the mammalian species, into the hydrophobic signal peptide that directs COX-1 into the lumen of the endoplasmic reticulum and nuclear envelope. Also provided in the present invention are COX-variant proteins expressed efficiently in insect cells as membrane-bound proteins. COX-3 and PCOX-1a are examples of COX-1 variants described herein. While the expression and activities of COX-3 and PCOX-1a are specifically described, it is noted that such expression is applicable to any of the COX-1 variants provided in the disclosure. Accordingly, expression of PCOX1b, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) or hCOX-3(cs) can be easily accomplished by the techniques described herein and by the knowledge of one skilled in the art of molecular biology.

[00235] Exemplary COX-3, but not PCOX-1a, possesses cyclooxygenase activity and this activity is dependent on the enzyme being glycosylated. Methods of screening for compounds that selectively inhibit COX-1 variants are also provided . For example, COX-1 variant activity can be inhibited by analgesic/antipyretic drugs such as acetaminophen, phenacetin, antipyrine, dipyrone and NSAIDs. Therefore, methods for identifying compounds useful for treating, for example, central nervous system disorders (e.g., decrease pain and/or fever) are provided.

[00236] By way of example only, but not by way of limitation, the invention provides specific compounds that target a COX-1 variant for inhibition. Methods of manufacturing derivatives of such compounds are well known to those skilled in the art of biochemistry. Thus, the invention encompasses derivatives of the disclosed compounds that are identified as possessing COX-1 variant inhibitory activity. Examples of COX inhibitors include Celecoxib (Celebrex®) and rofecoxib (Vioxx ®) which were developed as COX-2 selective

inhibitors. Other NSAIDs including meloxicam (Mobic®), nimesulide, and etodolac (Lodine ®), were also identified COX-2 preferential inhibitors. The invention provides a mechanism for identifying variants of the above-described compounds that preferentially inhibit a COX-1 variant over that of COX-1 and or COX-2.

[00237] Analysis by Northern blot of COX mRNAs isolated from a variety of canine tissues demonstrated that all RNA samples, with the exception of those from brain (cortex), contained primarily a single 2.6 kb mRNA that hybridized to a canine COX-1 cDNA probe. The canine probe was ~1.0kbp in size and was made to the coding region of the COX-1 mRNA. This probe used in these experiments was generated by reverse transcription-coupled polymerase chain reaction (RT-PCR) using degenerate oligonucleotides made to conserved sequences in COX isozymes. The identity of the probe was confirmed by DNA sequencing.. In contrast to what was observed in other tissues, this probe detected a novel COX-1 mRNA in total RNA isolated from brain tissue (Figure 5, panel A). This novel RNA was approximately 1.9 kb in size in contrast to the 2.6 kb mRNA that encodes COX-1 in dogs. A similarly sized message was detected in chicken brain mRNA (Figure 5, panel b).

[00238] Further Northern blot experimentation showed that the 1.9 kb mRNA was enriched in the poly A fraction of RNA and, therefore, was not likely a breakdown product of unprocessed RNA transcripts (Figure 5 panel c). Moreover, (RT-PCR) experiments demonstrated that this novel 1.9 kb COX-1-related mRNA was not the product of an alternative polyadenylation process which would change the length of the 3' untranslated region of the COX-1 RNA but otherwise leave the coding region unchanged. This was confirmed by Southern blot analysis of PCR generated fragment using probes specific for the 3' untranslated region of the message. In these experiment the same 3' untranslated region was found to be be present in both the 2.0 and 2.8 kb COX-1 RNAs. Therefore, the 1.9 kb RNA appeared to reflect changes in the coding region of a COX-1 mRNA or in a highly related mRNA.

[00239] To identify changes in the coding region present in the 1.9 kb RNA, cDNA libraries were created in both lambda ZIPLOX® and lambda ZAP® cloning vectors purchased from Gibco BRL and Stratagene, respectively. Methods of cDNA synthesis, vector ligation, and phage propagation are known to those skilled in the art. Approximately 45,000 recombinants from the ZIPLOX® library and ~149,000 recombinants from the lambdaZAP® libraries were probed with the canine 1.0 kb COX-1 cDNA fragment, radiolabeled with α^{32}PCTP and hybridized at a concentration of 2 X 10^6cpm/ml.

[00240] Eleven clones were isolated that strongly hybridized to the canine COX-1 clone and were subjected to DNA sequencing by automated DNA sequencing. All of these clones contained some DNA sequence that was essentially identical with canine COX-1 cDNA. However, three clones contained an insertion of 90 nucleotides of extra sequence near or at the 5' end of their respective cDNAs. The analysis of this inserted sequence found that it was located at the position where intron-1 of the canine COX-1 gene would be predicted to be located based on the position of this intron in mouse and human COX-1 genes. Moreover, it was determined that the retained 90-nucleotide sequence in these canine cDNAs showed striking sequence similarity to intron-1 in human and mouse COX-1 genes and contained 5' and 3' consensus splice sites indicative of a retained intron. The present study shows that these novel transcripts reflect an in-frame retention in the mRNA of intron-1 which would be predicted to cause significant changes in the biochemical properties of the COX-related protein encoded by these cDNAs.

[00241] Of the three intron-1 containing clone identified, one was found to contain, in addition to the 90-nucleotide insertion, an approximately 657 bp in-frame deletion. This in-frame deletion corresponded approximately to the removal of exons 5-8 of the COX-1 message. Additionally, one codon appears to be deleted from the 5' end of exon 4 and another codon appears to be added from the 3' end of exon 8.. This deletion is predicted to remove parts of the catalytic domain that might decrease peroxidase activity and might alter, but not destroy the fatty acid oxygenation activity of the enzyme encoded by this enzyme.

[00242] COX-1 variants (e.g. COX-3, PCOX-1a, hCOX-3(cc), hCOX-3(af), hCOX-3 (del10) and hCOX-3(cs)) contain an in-frame insertion of intron-1. Insertion of this sequence in these RNAs occurs 2 amino acid residues downstream from the initiating methionine of the protein and results in the addition of about 30 amino acids into the signal peptide. This addition may change the subcellular location of this enzyme in the cell. In particular, it is predicted to target this protein to specific organelles, such as the endoplasmic reticulum, nuclear envelope, lipid bodies or of the membrane structures in the cytoplasm where the enzyme would bind to cytosolic surfaces of cell membranes by virtue of its retained membrane binding domain. The location of these enzymes to the cytosolic surface of cellular membranes or lipid bodies would be predicted to alter folding of the protein since it would lack glycosylation provided by microsomal enzyme. Also the enzyme would contain approximately 60 amino acids at to its amino terminus that would not normally be in this protein. This addition, itself, would alter folding and likely prevent dimerization. The present data indicate that a COX-1 variant protein, although containing all or significant

portions of the COX-1 sequence, would have different enzymatic properties then those of the COX-1 proteins.

[00243] One example of a COX-1 variant includes PCOX-1a which exhibits a significant (219 amino acid) deletion in addition to having an insertion of intron -1 at its amino terminus. This enzyme possesses structural motifs for binding heme, having peroxidase activity, and oxygenating fatty acids similar to PIOXs. This means that, unlike COX-1 and COX-2, PCOX-1a will not likely produce products such as prostaglandins that contain cyclopentane rings, since formation of such products require interaction of the fatty acid with hydrophobic residues deleted in PCOX-1a. Instead, it is likely that PCOX-1a forms monooxygenated hydroperoxy- or hydroxy- derivatives of fatty acids similar to PIOXs.

[00244] The structure of the COX-1 gene is known in human and mouse. Analysis of the structure of intron-1 in these species show that both organisms contain an intron-1 that is similar in size to that in dogs which, when retained, would provide an in-frame insertion into the signal peptide encoding the protein (Figure 7). Four amino acids (ArgGluXAspPro) at the amino terminus of this intron are conserved in all three species. RNA's were analyzed by Northern blot using oligonucleotide antisense probes specific to intron 1. Canine tissues were analyzed with a oligonucleotide (50 bp in length) found in intron 1. It detected specific RNA species containing this intron in a variety of tissues, including extensively in brain (Figure 8). RNA isolated from a variety of human tissues were analyzed by Northern Blot using an anti-sense oligo (50 nucleotides in length) to intron-1. Washing conditions for this blot included two 30- minute washes at a temperature -20 degrees below the calculated melting temperature (Tm) of the probe. This was followed by one 10 minute wash at 4°C degrees below the calculated melting temperature. This high degree of stringency predicts an authentic rather than non-specific interaction of the probe with any interacting RNA. A 5.2 kb RNA was detected by the intron-1 specific oligonucleotide probe in human tissues of the forebrain, particularly the cortex, as well as in heart, muscle, liver, placenta, kidney and pancreas. The amygdala, hippocampus, whole brain, and lung and other tissues showed low expression of this RNA. In addition to the 5.2 kilobase transcript, several smaller RNAs of ~2-2.8 kb were detected by the probe in the cortex and other regions of the brain.

[00245] To further assure that intron-1 containing COX-1 transcripts exist in humans, reverse transcription coupled PCR was performed using RNA from human cells and tissues. Amplification of a predicted ~1.8kb fragment was achieved using a forward primer located in intron1 of the human gene and a reverse primer located immediately 3' to the predicted stop codon of the message. This amplicon hybridized in a Southern blot procedure with canine

COX-1 cDNA. In the Southern blot procedure the blot was washed at several times at 65°C in 2X SSC.

[00246] Antisense oligonucleotides to the first intron of human and canine cyclooxygenase (COX)-1 genes were synthesized and end-labeled using (γ-^{32}P)-dATP. A canine cerebral cortex cDNA library was screened using a ~1.0 kb canine COX-1 fragment by reverse transcription-coupled PCR (RT-PCR). The library was also screened with a ^{32}P-labeled canine COX-1 intron 1 antisense oligonucleotide. Two full-length clones were isolated, completely sequenced, and designated COX-3 and PCOX-1a. Both were derived from the canine COX-1 gene but retain intron 1. PCOX-1a also has a 657 bp in-frame deletion spanning exons 5-8.

[00247] Canine cerebral cortex cDNA was synthesized, and primers were designed for PCR amplification. The sense primer (5'-CGGATCCGCCGCCCAGAGCTATGAG-3' (SEQ ID NO:7)) corresponded to nucleotides 15-32 of canine COX-3 sequence (submitted to GenBank under accession no. AF535138), with the 3' end of the primer being 2 nucleotides downstream of the initiating methionine. The antisense primer (5'-CGCCATCCTGGTGGGGGTCAGGCAC ACGGA-3' (SEQ ID NO:8)) corresponded to nucleotides 1865-1894, located 32 nucleotides upstream of the stop codon.

[00248] Northern blot analysis of human tissues with an intron-1 probe detected an ~5.2 kb mRNA. Marathon-readyTM human cerebral cortex cDNA (Clontech) was amplified by PCR (Clontech - Advantage® 2 PCR enzyme system) using 5' and 3' primers, and a ~4.2 kb amplified fragment was recovered and found to contain the entire coding region of human COX-1 with intron 1 retained.

[00249] Both COX-3 and PCOX-1a were cloned into the baculovirus expression vector pBlueBac 4.5/V5-His (Invitrogen). Sf9 cells (~1 x 10^6) were infected with viral stocks at a multiplicity of infection (MOI) of 3 for expression of COX-3, PCOX-1a, mouse COX-1 and mouse COX-2.

[00250] Total protein (20 μg) from human aorta was analyzed by Western blotting, using COX-1 monoclonal antibody (MAb) (Cayman Chemical, Ann Arbor, MI) and COX-3 antipeptide polyclonal antibodies (PAb). Primary antibodies were either preincubated with a mixture of human and mouse COX-1 intron 1 peptide (described below) for 1 hr, 4°C, or left unblocked. Blots were processed with appropriate rabbit-anti-mouse secondary antibody (1:2000) or goat-anti-rabbit secondary antibody (1:10,000) from Sigma. Densitometry of the autoradiographic image was performed using the AlphaImager™ 2000 Documentation and Analysis System (Alpha Innotech Corporation).

[00251] Tunicamycin was added to a final concentration of 10 μg/ml to insect cells 1 hr after infection with baculovirus constructs. The cells were cultured and harvested after 48 hr. COX activity of intact cells was determined by radioimmunoassay (RIA) (Salmon, 1978). COX activity in intact Tu-treated cells was compared with activity in untreated cells infected with the appropriate virus (MOI = 3).

[00252] Sf9 cells were infected with a high titer viral stock at a MOI of 3 and cultured for 48 hr. Infected cells expressing COX-3 were aliquoted into tubes (~1.5×10^6 cells) and centrifuged (1000 × g, 5min). The supernatant was discarded and the cell pellet resuspended in 100 μl serum-free media containing the drug to be tested and preincubated at room temperature for 30 minutes. Arachidonic acid (100 μl, final concentration 5 or 30 μM) was then added, mixed and incubated (37°C, 10min). Samples were then centrifuged and 100 μl of the supernatant was assayed for COX activity by RIA for PGE_2. Assays were performed multiple times in triplicate. Inhibition curves were constructed and IC_{50} values were determined using Prism® 3.0 (GraphPad, San Diego).

[00253] Peptides corresponding to the first 13 amino acids of human and mouse COX-3 primary sequence, as predicted by genomic clone sequences, were synthesized and coupled to keyhole limpet hemocyanin. A mixture of the human (MSRECDPGARWGC (SEQ ID NO:20)) and mouse (MSREFDPEAPRNC (SEQ ID NO:21)) peptides were injected into New Zealand white rabbits. The resulting polyclonal antibodies were then affinity purified using the above peptides immobilized on a Sulfolink™ coupling gel (Pierce) according to the manufacturer's instructions.

[00254] Northern blot analysis detected a ~5.2 kb mRNA containing intron 1 (Fig. 10, panel B). The antisense primer (HCLE: 5'-CGGATCCTGGA ATAGGCCACCGGATGGAAGGA-3' (SEQ ID NO:9)) was designed from the 3' end of the published sequence and the sense primer (HCF: 5'-CGGATCCTGCGTCCC GCACCCCAGCA-3' (SEQ ID NO:17) from a site 5 nucleotides upstream from the initiation codon of the human COX-1 gene (Gen-Bank, accession number L 08404). The primers were designed with a *Bam*HI recognition sequence at their 5' ends to facilitate cloning.

[00255] Marathon-ready™ human cerebral cortex cDNA (Clontech) was amplified by PCR (Clontech - Advantage® 2 PCR enzyme system) using the above primers. The resulting ~4.2 kb amplified fragment was recovered from a low melting agarose gel and reamplified using nested primers (sense, HCEI: 5'-CGGATCCGCGCCATGAGCC GTGA-3' (SEQ ID NO:18); antisense, HCS: 5'-CGGATCCTCAGAGCTCTGTGGATGGTCGCT-3' (SEQ ID

NO:19). The resulting fragment (~2.0 kb) containing the entire coding region of human COX-1 (with intron 1 retained) was then cloned in the plasmid Bluescript and sequenced.

[00256] Two distinct mRNA species (~2.6 and ~1.9 kb) were detected on a Northern blot with a canine COX-1 coding region cDNA probe utilizing RNA isolated from canine cerebral cortex (Fig. 10, panel A, lane 1). To further investigate these transcripts, a canine cerebral cortex cDNA library was constructed and the non-amplified library was screened as described above. Eleven clones were isolated and subsequently characterized by automated DNA sequencing. All of the eleven clones were found to contain canine COX-1 cDNA sequence. However, three clones harbored an insertion of 90 nucleotides at, or near, the 5' end of their respective cDNAs, which showed 75% sequence identity to intron 1 of either human or mouse COX-1 genes. This extra sequence also contained 5' and 3' consensus splice sites indicative of a retained intron. In addition to the retention of intron 1, one of the three clones had a 657 bp in-frame deletion corresponding to exons 5-8 of the COX-1 message.

[00257] To determine whether the two previously detected COX mRNA transcripts (i.e. ~2.6 and ~1.9 kb) harbored intron 1, the Northern blot experiment was repeated utilizing a radiolabeled antisense canine COX-1 intron 1-specific oligonucleotide probe (CCI) (Fig. 10, panel A, lane 2). Importantly, the ~1.9 kb mRNA transcript and the ~2.6 kb transcript were detected suggesting that novel COX-1 mRNA splice variants were indeed expressed in canine cerebral cortex. Therefore the novel COX cDNA clone, which harbored a non-spliced intron 1 and corresponded to the ~2.6 kb mRNA transcript has been designated as COX-3. Additionally, the novel COX cDNA clone which harbored intron 1, lacked exons 5-8, and corresponded to the ~1.9 kb mRNA transcript, has been designated partial COX-1a or PCOX-1a (Fig. 11).

[00258] Reverse transcription-coupled PCR of canine cerebral cortex RNA as well as analysis of Northern blots, indicated that COX-3 mRNA is present in this brain region at about 5% of the level of COX-1 mRNA (Fig. 10, panel A). Interestingly, these analyses also demonstrated that the ~1.9 kb mRNA corresponding to PCOX-1a was actually a mixture of two mRNAs that differed in size by ~90 nucleotides (Fig. 10, panel B). One of these mRNAs was PCOX-1a and the other (PCOX-1b) was identical to PCOX-1a except that PCOX-1b lacked intron 1. PCOX-1a and PCOX-1b are expressed in equal amounts in brain cortex (Fig. 10, panel B).

[00259] To determine whether novel COX-1 related mRNA transcripts were also expressed in human tissues, human Northern blot experiments were performed utilizing a human-intron-1 specific (HCI) probe. Importantly, these results demonstrated the existence of

a novel ~5.2 and ~2.8 kb mRNA transcripts (Fig. 10, panel C). Faint hybridization signals were also seen around 1.9 kb. Hybridization of HCI to the ~5.2 kb form was tissue-specific, with highest levels present in the cerebral cortex followed by the heart. These observations differ from the characterized expression patterns of COX-1 mRNA.

[00260] COX enzymes are intralumenal residents of the endoplasmic reticulum and depend on N-linked glycosylation for proper folding and activity. Retention of intron 1 could prevent COX-3 and PCOX-1 expression by preventing export of these mRNAs from the nucleus or by targeting these proteins to another subcellular compartment, preventing glycosylation. Therefore, insect cells (Sf9) were infected with recombinant baculovirus expressing COX-3, PCOX-1, and COX-1 and cell homogenates were assayed for protein expression by Western blotting. Antibodies specific for the conserved amino acid sequence (MSREXDPXA) predicted to be encoded by intron 1 in mammals were used to probe for COX-3 and PCOX-1a and b. This analysis demonstrated that both COX-3 and PCOX-1 are efficiently expressed in insect cells. No detectable products resulting from removal of intron 1 by splicing were detected immunologically or by RT-PCR analysis of RNA extracted from infected Sf9 cells. Moreover, the signal peptide, which in COX-3 and PCOX-1a or b contains an additional intron 1 encoded sequence, was not removed by signal peptidase as it is in COX-1 and COX-2.

[00261] Posttranslational N-linked glycosylation of COX-3 and PCOX-1 was compared to that of COX-1 using tunicamycin to inhibit core glycosylation. Immunoblot analysis demonstrated a decrease in or disappearance of glycosylated forms of COX-3, PCOX-1, and COX-1 (Fig. 12 Top; left, middle and right panels, respectively). Expression systems were then assayed for cyclooxygenase activity by measuring the production of PGE_2 in whole insect cells. COX-3 activity was found to be ~20% of that of COX-1 and PCOX-1 completely lacked detectable COX activity (Fig. 12 bottom panels). COX activity in cells treated with tunicamycin was found to be significantly decreased or abolished by this drug, indicating that N-linked glycosylation is necessary for COX activity of COX-3.

[00262] RNA studies in human tissues indicated highest levels of COX-3 message to be in the cerebral cortex and heart. Western blot analysis of human aorta (Fig. 15) using either COX-1 monoclonal antibody or COX-3 antipeptide polyclonal antibody detected the presence of distinct 65 and 53 kDa COX-1 related proteins. Additionally, the COX-1 but not COX-3 antibody, detected a 69 kDa protein, corresponding to glycosylated COX-1, as well as a 50 kDa protein, which may represent a proteolytic fragment of COX-1 or PCOX-1b. Detection of both of the 65 and 53 kDa proteins was selectively reduced by preincubation of

the anti-peptide sera with its cognate peptide, whereas detection of the same proteins by the COX-1 monoclonal antibody was unaffected by this treatment.

[00263] Analgesic/antipyretic drugs and NSAIDs were tested for their ability to inhibit COX activity of COX-3 as compared to their ability to inhibit COX-1 and COX-2. Analyses were done in the presence of exogenously added arachidonic acid at 30 and 5 μM concentrations. At the higher concentration of substrate, only COX-3 was inhibited by acetaminophen (Fig. 13, panel A). Moreover, COX-3 was found to be significantly more sensitive to acetaminophen than either COX-1 or COX-2 at the lower substrate concentration (Fig. 13, panel B). Acetaminophen inhibited COX-3 at an IC_{50} value of 64 μM when done in the presence of 5 μM arachidonic acid, whereas IC_{50} values for COX-1 and COX-2 were 2.1- and 92.4-fold higher, respectively.

[00264] Acetaminophen is considered to be the active metabolite of phenacetin, a once popular analgesic/antipyretic drug that is no longer extensively used due to the occurrence of methemoglobinemia, renal toxicity, and suspected renal and bladder carcinogenesis. Phenacetin is rapidly O-de-ethylated in the body to form acetaminophen and is further metabolized to other minor but toxic compounds. Thus only small levels of of phenacetin circulate in the blood. Interestingly, however, phenacetin was much more potent at inhibiting COX-3 than was acetaminophen (Fig. 13, panel C). Under substrate conditions of 30 μM, phenacetin inhibited COX-3 at an IC_{50} value of 102 μM as opposed to 460 μM for acetaminophen tested under similar conditions. As with acetaminophen, phenacetin preferentially inhibited COX-3.

[00265] Another analgesic/antipyretic drug, dipyrone, was also significantly more potent at inhibiting COX-3 than either COX-1 or COX-2 (Fig. 13, panel D). Dipyrone inhibited COX-3 with an IC_{50} value of 52 μM and COX-1 at a 6.6-fold higher concentration. No detectable inhibition of COX-2 by dipyrone was observed below 1 mM. Dipyrone is a pro-drug that spontaneously breaks down in aqueous solutions to a variety of structurally related pyrazolone compounds that differ in their potency as analgesic/antipyretic agents. Antipyrine and dimethylaminopyrene are similar to two breakdown products of dipyrone, and possess markedly reduced therapeutic potency and similarly show markedly reduced inhibition of COX-3 as compared to dipyrone (Table 1). However, these compounds, like other analgesic/antipyretic agents, preferentially inhibit COX-3.

[00266] COX-3 was also found to differ in its sensitivity to inhibition by a selection of NSAIDs. Diclofenac was the most potent inhibitor of COX-3 tested and diclofenac, aspirin

and ibuprofen preferentially inhibited COX-3 over COX-1 and COX-2. The IC_{50} values of these drugs are tabulated (Table 1). Importantly, the overall results indicate that COX-3 possesses a COX activity which differs pharmacologically from both COX-1 and COX-2.

[00267] Both COX-3 and PCOX-1a are formed by intron retention. We have previously shown that COX-2 in chicken is regulated by intron 1 retention, similar to that seen with COX-3. Unspliced mRNAs are largely retained in the nucleus. In the case of chicken COX-2, retention of intron 1 prevents translation and nuclear export of the mRNA. However, both COX-3 and PCOX-1a mRNAs in insect cells retain the intron and are exported from the nucleus and are translated (Fig. 12). The polypeptides produced from COX-3 and PCOX-1a include sequence encoded by the intron 1 and are functionally different from fully-spliced COX-1. Therefore retention of intron 1 provides a mechanism by which a novel COX enzyme, COX-3, can be produced in cells and tissues. Consistent with the concept that retention of intron 1 is important in creating COX-3 and/or regulating COX-1 is the finding that the DNA sequence of intron 1 from dog, human, and mouse COX-1 genes displays a high degree of conservation. This is most evident in the 5' and central regions of the intron. Overall intron 1 shows 41% sequence identity between all three species with the sequence 5'-GCCTcNGGNGGAGCCTYGAAYGCYAG-3' (SEQ ID NO:44) in the central region of the intron being highly conserved. In fact intron 1, is more conserved in these species than is exon 1, suggesting that intron 1 plays an important and similar role in mammals. Highly conserved elements of intron 1 may also play a role in regulation of its retention. Further buttressing the concept that intron 1 plays an important role in regulating COX-3 expression is the fact that the gene structure of COX-1 and COX-2 differ only in their placement of intron 1. COX-1 has 10 introns while COX-2 has 9. The additional intron in the COX-1 gene is intron 1, which is retained in COX-3.

[00268] COX-3 shares all the catalytic features and important structural features of COX-1 and COX-2. However, the insertion of intron 1, two amino acids downstream from the initiating methionine would result in the addition of 30 amino acids to the signal peptide. Despite having a signal peptide and intron-1-encoded sequence retained, COX-3 co-migrates with COX-1 in SDS-PAGE gels. It also appears to enter the endoplasmic reticulum where it is glycosylated and its glycosylation is required for activity. In insect cells COX-3 shows approximately 20% of the activity of COX-1, which in turn exhibits about 20% of the activity of COX-2. COX-1, COX-2, COX-3, and PCOX-1a all show equivalent expression in our baculovirus system, and so a lowered ability of insect cells to express active COX-1 relative to COX-2 may be due to the inability of insect cells to posttranslationally process COX-1

correctly. Subcellular localization studies done by differential centrifugation demonstrate that neither COX-3 nor PCOX-1a is cytosolic. Membrane binding of both proteins is predicted from the fact that both retain a membrane binding domain and both appear to enter the lumen of the endoplasmic reticulum. Retention of intron 1 could alter folding and may affect dimerization and the active site. These effects could be through structural changes or altered protein targeting. COX-1 site-directed mutagenesis of either Cys^{313} or Cys^{540}, both of which are more than 25 Å from the heme iron, was observed to reduce the activity of the enzyme by 80-90%. Therefore, although COX-3 contains all of the COX-1 sequence, the retained intron sequence could significantly alter its enzymatic properties. The present inhibition studies of COX-3 indicate this to be the case.

[00269] The present studies shows that the COX-1 variant COX-3 is sensitive to drugs that are analgesic/antipyretic, but which have low anti-inflammatory activity. Pain and fever have many etiologies that employ complex cellular and biochemical pathways. The finding that COX-3 is sensitive to analgesic/antipyretic drugs suggests that the COX-1 gene plays an integral role in pain and/or fever. Depending on the physiological context, pain pathways involve products from either the COX-1 or COX-2 genes. COX-2 selective drugs, for example, are clinically useful in inhibiting inflammatory pain in humans and are more potent than COX-1-selective NSAIDs at inhibiting pain induced by pro-inflammatory agents (e.g. carrageenan) in some paw inflammation assays in rodents. COX-1 selective drugs, in contrast, are superior to COX-2-selective agents at inhibiting visceronociception caused by a variety of chemical pain stimulators. Moreover, Ballou et al. (*Proc. Natl. Acad. Sci. USA* 97:10272, 2000) found that visceronociception was greatly decreased in COX-1 but not COX-2 knockout mice. Both COX-1 and COX-2, on the other hand, have been implicated in nociception models that measure analgesia outside the gut, such as in formalin and urate crystal tests. A role for COX-1 in pain is further supported by the fact that COX-1-selective NSAIDs (e.g. aspirin, ketorolac, ketoprofen, ibuprofen, and suprofen – are clinically important analgesic agents in humans and animals). Despite their relative exclusion from the brain, these drugs may reach sufficient concentration to effect COX-3 in the brain. Furthermore, the analgesic effects of these drugs often occur at significantly lower doses than those needed to inhibit inflammation. Clinical and experimental association of COX-1 and pain may be functionally explained by the finding that COX-1 is a marker for subpopulations of putative nociceptor neurons in the dorsal root ganglion.

[00270] With regard to pyresis, COX-2 but not COX-1 knockout mice demonstrate reduction in LPS- and interleukin-1-induced fevers, and some new COX-1 selective

inhibitors, such as SC-560, have proven ineffective at inhibiting LPS-induced fever in animal models. Clinically, rofecoxib, a COX-2 selective inhibitor, inhibits naturally occurring fever and also inhibits the maintenance of fever in animal models. Yet aspirin, a COX-1 preferential inhibitor is one of the most effective antipyretic NSAIDs, and inhibits fever at doses ranging from 5-15 mg/kg, far below the 60-80 mg/kg used to treat inflammatory disease. Furthermore, nimesulide, a COX-2 preferential inhibitor, was found to be antipyretic in dogs only at plasma concentrations that would also inhibit COX-1. Thus a role for COX-1 in fever may exist.

[00271] The mechanism of action of acetaminophen has been unknown and postulated to be through inhibition of a brain COX that has never been identified. Northern blot analysis and cDNA cloning show that COX-3 is expressed in canine brain. COX-3 also appears from Northern blot studies (Fig. 10) to be expressed in specific regions of the human brain, in particular cerebral cortex. Moreover, our studies using ectopically expressed COX-3 in insect cells demonstrate that COX-3 is significantly more sensitive to acetaminophen than COX-1 and COX-2. Under physiological conditions, where steady-state acetaminophen concentrations reach approximately 100 μM, and where free arachidonic acid levels are 1-5μM, only COX-3 is predicted to be appreciably inhibited. These findings suggest that inhibition of COX-3 in brain and the spinal cord could be the long sought-after target for acetaminophen.

[00272] The proposed mechanism of action for acetaminophen inhibition of COX-3 also appears to extend to pyrazolone drugs such as dipyrone and related compounds aminopyrine and antipyrine. Dipyrone is a potent analgesic/antipyretic drug which, like acetaminophen, lacks antiinflammatory activity. Dypyrone however is structurally unrelated to acetaminophen. Consistent with COX-3 being the target for analgesic and antipyretic drugs is the finding that these structurally dissimilar agents preferentially inhibit COX-3 and that their therapeutic potencies follow their ability to inhibit COX-3. The IC_{50} values are as follows: dipyrone (52 μM), 4-dimethylaminoantipyrine (688 μM), and antipyrine (862 μM). Only dipyrone is therapeutically effective as an analgesic/antipyretic drug. Its active breakdown product, 4-methylaminoantipyrine reaches concentrations of 104 μM and 86 μM in plasma and the central nervous system, respectively. Thus, COX-3 inhibition occurs at known physiological concentrations of pyralazone drugs as well as acetaminophen. Additionally, inhibition of COX-3 does not require addition of glutathione, epinephrine, or other exogenously added "cofactors" which have been required in other systems.

[00273] Analgesic/antipyretic drugs inhibit COX-3 activity at higher concentrations than standard NSAIDs. From a therapeutic standpoint this may be rationalized by the fact that these drugs penetrate the blood brain barrier well, thus accumulating in the CNS at high enough concentrations to inhibit COX-3. Analgesic/antipyretic drugs, like acetaminophen, have long been postulated to have a central mechanism of action. Carboxylate-containing NSAIDs, on the other hand, cross the blood-brain barrier poorly and have a well-defined ability to reduce pain peripherally by reducing prostaglandin synthesis that sensitizes nociceptors. Several central analgesic mechanisms of action for NSAIDs have also been proposed wherein inhibition of prostaglandin synthesis in brain or spinal cord, potentially via COX-3, could contribute to the analgesic action of NSAIDs. Thus, action of NSAIDs in the body is likely to be central (COX-3) as well as peripheral (COX-1, COX-2 and COX-3). COX-1 variants in the CNS may be an essential target of both analgesic/antipyretics and standard NSAIDs.

[00274] A comparison of COX-3 inhibition by analgesic/antipyretics and NSAIDs in these studies (Fig. 13 and Table 1) suggest that both types of drugs may be capable of modulating a COX-variant activity (e.g., COX-3). Table 1 shows IC50 values of selected analgesic/antipyretic drugs and NSAIDs. Also shown are relevant inhibition ratios of COX-1/COX-3 and COX-2/COX-3 indicating preferential inhibition towards COX-3. All assays were carried out at 30 mM arachidonic acid. Asterisks in the table indicate the following notations: *, 4-dimethylaminoantipyrine; **, no detectable inhibition at 1mM; ***, ratios not applicable.

Table 1

DRUG	IC_{50}, µM		
	COX-1	**COX-2**	**COX-3**
Acetaminophen	>1000	>1000	460
Aminopyrine*	>1000	>1000	688
Antipyrine	>1000	>1000	863
Aspirin	10	>1000	3.1
Diclofenac	0.035	0.041	0.008
Dipyrone	350	>1000	52
Ibuprofen	2.4	5.7	0.24
Indomethacin	0.010	0.66	0.016
Phenacetin	>1000	>1000	102
Caffeine	>1000	>1000	>1000
Thalidomide	>1000	>1000	>1000

[00275] Comparison of COX-1, COX-2 and COX-3 demonstrated that COX-3 is most like COX-1 in its sensitivity to NSAIDs but is significantly more sensitive to many drugs, such as diclofenac and ibuprofen, and less sensitive to others like sodium salicylate than is either COX-1 or COX-2. The higher sensitivity of COX-3 to NSAID inhibition may allow it to be preferentially inhibited in the CNS by low levels of NSAIDs that cross the blood-brain barrier. Furthermore, the differential sensitivity of COX-3 to analgesic/antipyretic drugs and NSAIDs suggests that highly selective inhibitors can be made for COX-3.

[00276] Human COX-3 is mainly expressed as a ~5.2 kb mRNA and has a tissue-specific pattern of expression (Fig. 10, panel C). This ~5.2 kb mRNA is an alternatively polyadenylated human COX-1 message previously reported and partly characterized in its 3' region (5). It appears, therefore, that the retention of intron 1 may influence the site at which the mRNA is polyadenylated. This finding suggests that the 3' untranslated regions of the mRNA may play a functional role in expression of COX-3 and perhaps PCOX-1a. The functional significance and the mechanism by which intron retention and alternative polyadenylation are coordinated need to be elucidated. It is also interesting to note that the ~5.2 kb mRNA has been shown to be regulatable (36) and hence may be regulated in response to physiological stimuli and signal transduction. Indeed, the levels of COX-3 mRNA in human and canine cerebral cortex are relatively low. This may be due to cell type-

specific expression such as has been shown for COX-1 immunoreactive protein in a subpopulation of putative nociceptor neurons (23). However, COX-3 in human will require further experimentation since some of the published sequences differ by one nucleotide in intron 1 and hence are out of frame. These may constitute genuine polymorphisms or sequencing errors. Alternatively, intron 1 may be out of frame in humans, requiring other mechanisms such as ribosomal frame shifting to produce a functional COX-3 protein.

[00277] The present studies have identified novel COX-1 variants, including COX-3 protein of about 65 kDa in human aorta and a PCOX-1a protein of about 53 kDa. These proteins are detected by both COX-3 antipeptide polyclonal antibody and a COX-1 monoclonal antibody and appear to be present at about 25% of the level of COX-1. The 65 kDa protein is smaller than would be predicted if the protein is glycosylated to the same extent as COX-1, suggesting hypoglycosylation or other differences exist between the 65 kDa protein and COX-1. The 53 kDa proteins are present as a doublet, and are of a higher molecular weight than that predicted by the PCOX-1a protein primary sequence. This suggests that, like canine PCOX-1a expressed in insect cells, the human protein may be glycosylated, and that different glycosylation states may exist giving rise to the doublet observed. A 50 kDa protein is also detected only by the COX-1 monoclonal antibody, and is a candidate for being PCOX-1b. It appears to be present at about 15% of the level of COX-1.

[00278] PCOX-1a is identical to COX-3 except for a deletion of 219 amino acids in the catalytic domain of the protein, corresponding to exons 5-8. It lacks detectable cyclooxygenase activity, as shown by its inability to make prostaglandins from arachidonic acid. The deleted portion contains structural helices HE, H1, H2, H3, H5, and part of H6 defined for COX-1 and COX-2. Of these helices, H2 and H5 form part of the core peroxidase catalytic site. Because of the lack of H2 and H5, PCOX-1 most likely lacks detectable peroxidase activity. In this way it is similar to plant PIOX enzymes and *Gaeumannomyces graminis* linoleate diol synthase (LDS), which also lack peroxidase activity. They do, however, have fatty acid oxygenase activity that is similar in mechanism to the oxygenase activity of cyclooxygenase, and contain sequences similar to those found in COX-1 and -2. Figure 14 shows an alignment of H2, H5, and H8 (helix containing Tyr^{385} and the proximal histidine) from the consensus sequences of COX-1 and -2 with PIOXs and LDS.

[00279] Although the peroxidase activity of cyclooxygenase is needed to create the protein radical used in the cyclooxygenase reaction, continued peroxidase activity is not essential for continued cyclooxygenase activity. After the enzyme has been primed by one

peroxidase reaction, it can continue to catalyze oxygenation of the substrate, because the tyrosine radical is regenerated after each oxygenation reaction. This priming mechanism for cyclooxygenase also seems to work with PIOXs and LDS. The active site tyrosine and proximal histidine of cyclooxygenase are conserved in PIOXs and LDS. It is believed that these enzymes have a reaction mechanism similar to COX, based on their similarity with cyclooxygenases. It is likely, then, that they do retain a low level of peroxidase activity that, although undetectable, is sufficient to prime them for oxygenase activity.

[00280] Because only one turnover of the peroxidase active site is required for cyclooxygenase activity in COX-1 and -2, there may be enough residual peroxidase activity in PCOX-1 proteins to prime them. However, we have shown that PCOX-1a does not have cyclooxygenase activity. It is possible, based on comparison with PIOXs that PCOX-1 proteins do retain lipid oxygenase activity, similar to the lipid oxygenase activity of PIOXs. Further studies are required to determine what the substrate of PCOX-1 proteins would be.

[00281] Because PCOX-1a and PCOX-1b are missing such a large portion of its catalytic domain, it is possible that it needs to be bound to another protein for it to be catalytically active. We have previously found that cyclooxygenases bind to nucleobindin (Ballif, 1996). Nucleobindin is a candidate for binding to PCOX-1 proteins as well. Additionally, a form of COX-1 has been described that co-localizes with prostacyclin synthase in filamentous structures of cultured endothelial cells. This filamentous form of COX-1 has no cyclooxygenase activity, and is a candidate for being a PCOX-1 protein.

[00282] Human COX-3 is mainly expressed as a ~5.2 kb mRNA and has a tissue-specific pattern of expression (Fig. 10, panel C). This ~5.2 kb mRNA is an alternatively polyadenylated human COX-1 message and partly characterized its 3' region. The data provided herein indicates that retention of intron 1 influences the site at which the mRNA is polyadenylated. The functional significance and the mechanism by which intron retention and alternative polyadenylation are coordinated need to be elucidated. It is also interesting to note that the ~5.2 kb mRNA has been shown to be inducible and may be regulated in response to physiological stimuli and signal transduction. Indeed, the levels of COX-3 mRNA in human and canine cerebral cortex are relatively low and may be due to cell type-specific expression requiring specific signals. However, COX-3 in human will require further experimentation since some of the published sequences differ by one nucleotide in intron 1 and hence are out of frame. These may constitute genuine polymorphisms or sequencing errors. Alternatively, intron 1 may be out of frame in humans, requiring other mechanisms such as ribosomal frame shifting to produce a functional COX-3 protein. The

finding that retention of intron 1 is coordinately tied to alternative polyadenylation suggests that the 3' untranslated regions of the mRNA may play a functional role in expression.

[00283] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising intron 1, or fragment thereof, of cycloxygenase type 1, wherein the nucleic acid molecule is RNA.

2. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is mRNA.

3. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule cDNA.

4. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule encodes a polypeptide comprising at least one domain that catalyzes the cyclization and/or oxygenation of an fatty acid radical, at least one membrane-binding domain, and at least one heme binding domain.

5. The nucleic acid of claim 1, wherein the nucleic acid molecule encodes a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

6. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 or SEQ ID NO:13.

7. An isolated nucleic acid molecule selected from the group consisting of:
    a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1;
    b) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:3;
    c) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:4;
    d) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:6;
    e) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:10;

f) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:11;

g) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:12; and

h) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:13;

8. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2; and

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:5

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:14;

a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:15; and

a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:16.

9. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or 3 under stringent conditions; and

b) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:5, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:4 or 6 under stringent conditions.

10. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% homologous to the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 10, 11, 12 or 13 or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 200 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 10, 11, 12 or 13 or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 60% homologous to the amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16; and

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16, wherein the fragment comprises at least 15 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16.

11. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 7, 8, 9 or 10 under stringent conditions.

12. An isolated nucleic acid molecule comprising a nucleotide sequence which is complementary to the nucleotide sequence of the nucleic acid molecule of any one of claims 7, 8, 9 or 10.

13. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 7, 8, 9 or 10, and a nucleotide sequence encoding a heterologous polypeptide.

14. A vector comprising the nucleic acid molecule of any one of claims 1, 7, 8, 9 or 10.

15. The vector of claim 14, which is an expression vector.

16. A host cell transfected with the vector of claim 14.

17. A method of producing a polypeptide comprising culturing a host cell transfected with the vector of claim 14 in an appropriate culture medium to, thereby, produce the polypeptide.

18. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, 5, 14, 15 or 16;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO: 1, 3, 4, 6, 10, 11, 12 or 13 under stringent conditions;

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60 % homologous to a nucleic acid comprising the nucleotide sequence of SEQ ID NO: 1, 3, 4, 6, 10, 11, 12 or 13; and

d) a polypeptide comprising an amino acid sequence which is at least 60% homologous to the amino acid sequence of SEQ ID NO: 2, 5, 14, 15 or 16.

19. The polypeptide of claim 18 comprising the amino acid sequence of SEQ ID NO:2, 5, 14, 15 or 16.

20. The polypeptide of claim 18, further comprising heterologous amino acid sequences.

21. An antibody which selectively binds to a polypeptide of claim 18.

22. A method for detecting the presence of a polypeptide of claim 18 in a sample comprising:

a) contacting the sample with a compound which selectively binds to the polypeptide; and

b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 18 in the sample.

23. The method of claim 22, wherein the compound which binds to the polypeptide is an antibody.

24. A kit comprising a compound which selectively binds to a polypeptide of claim 18 and instructions for use.

25. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 7, 8, 9 or 10 in a sample comprising:
   a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and
   b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample to thereby detect the presence of a nucleic acid molecule of any one of claims 1, 7, 8, 9 or 10 in the sample.

26. The method of claim 25, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

27. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of any one of claims 1, 7, 8, 9 or 10, and instructions for use.

28. A method for identifying a compound which binds to a polypeptide of claim 18 comprising:
   a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and
   b) determining whether the polypeptide binds to the test compound.

29. The method of claim 28, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:
   a) detection of binding by direct detection of test compound/polypeptide binding;
   b) detection of binding using a competition binding assay; and
   c) detection of binding using an assay for activity.

30. A method for modulating the activity of a polypeptide of claim 18 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

31. A method for identifying a compound which modulates the activity of a polypeptide of claim 18 comprising:

a) contacting a polypeptide of claim 18 with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

32. A method for preventing or treating Alzheimer's Disease in a subject, said method comprising administering to said subject an effective amount of a specific inhibitor of COX-1 variant activity, in a pharmaceutically acceptable carrier.

33. The method as in claim 32 in which said specific inhibitor of COX-1 variant activity is a specific inhibitor of COX-1 variant activity encoded by a nucleic acid as set forth in claims 1, 7, 8, 9 or 10.

34. A method for ameliorating a neurodegenerative condition in a subject, said method comprising administering to said subject a specific inhibitor of cyclooxygenase type 1 activity encoded by a nucleic acid as set forth in claims 1, 7, 8, 9 or 10, in a pharmaceutically acceptable carrier.

35. The method as in claim 34 wherein said neurodegenerative condition is stroke, cerebral ischemia, a demyelinating condition, or mechanical injury.

36. A method for treating immune or inflammatory conditions associated with Alzheimer's Disease in a subject, said method comprising administering to said subject a specific inhibitor of a COX-1 variant activity, in a pharmaceutically acceptable carrier.

37. The method as in claim 36 in which said specific inhibitor of cyclooxygenase activity is a specific inhibitor of cyclooxygenase type 1 activity encoded by a nucleic acid as set forth in claims 1, 7, 8, 9 or 10.

38. A method of modulating expression, production, or formation of amyloid precursor protein (APP) in a subject, comprising administering to said subject an effective amount of an antagonist, wherein said antagonist is a non-steroidal anti-inflammatory

agent that is a specific inhibitor of a COX-1 variant, in a pharmaceutically acceptable carrier.

39. The method as in claim 38 in which said specific inhibitor of cyclooxygenase activity is a specific inhibitor of cyclooxygenase type 1 activity encoded by a nucleic acid as set forth in claims 1, 7, 8, 9 or 10.

40. A method for selectively inhibiting COX-3 or PCOX-1a activity in a subject, comprising administering a compound that selectively inhibits activity of the COX-3 or PCOX-1a gene product to a subject in need of such treatment.

41. The method of claim 40 in which the compound is a non-steroid anti-inflammatory compound.

42. A method for selectively inhibiting COX-3 or PCOX-1a activity in a subject, comprising administering a non-steroidal compound that selectively inhibits activity of the COX-3 or PCOX-1a gene product in a subject in need of such treatment, wherein the activity of the non-steroidal compound does not result in significant toxic side effects in the subject.

43. A method for selectively inhibiting COX-3 or PCOX-1a activity in a subject, comprising administering a non-steroidal compound that selectively inhibits activity of the COX-3 or PCOX-1a gene product in a subject in need of such treatment, wherein the ability of the non-steroidal compound to selectively inhibit the activity of the COX-3 or PCOX-1a gene product is determined by:

a) contacting a genetically engineered cell that expresses COX-3 or PCOX-1a, and not COX-1 or COX-2, with the compound and exposing the cell to a pre-determined amount of arachidonic acid;

b) contacting a genetically engineered cell that expresses COX-1 or COX-2, and not COX-3 or PCOX-1a, with the compound and exposing the cell to a pre-determined amount of arachidonic acid;

c) measuring the conversion of arachidonic acid to its prostaglandin metabolite; and

d) comparing the amount of the converted arachidonic acid converted by each cell exposed to the compound to the amount of the arachidonic acid converted by control cells that were not exposed to the compound, so that the compounds that inhibit COX-3 or PCOX-1a and not COX-1 or COX-2 activity are identified.

44. A method of identifying a compound that modulates the activity of COX-1 variant, the method comprising:

a) providing a cell transfected with a DNA encoding a COX-1 variant, wherein the cell expresses COX-1 variant;

b) contacting said cell, in an intact or disrupted state, with a test compound; and

c) determining whether the activity of COX-1 variant is decreased or increased in the presence of the test compound, wherein a decrease or increase in said COX-1 variant activity is an indication that the test compound modulates the activity of a COX-1 variant.

45. The method of claim 44, wherein the COX-1 variant is selected from the group consisting of COX-3, PCOX-1a), hCOX-3(cs1), hCOX-3(cs2) and hCOX-3(cs3).

46. The method of claim 44, wherein the DNA encoding a COX-1 variant encodes an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

47. The method of claim 44, wherein the DNA encoding a COX-1 variant is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 an SEQ ID NO:13.

48. The method of claim 44, wherein the cell is a mammalian cell.

49. The method of claim 44, wherein the cell is an insect cell.

50. The method of claim 44, wherein the cell is bacterial cell.

51. The method of claim 44, wherein the test compound is a non-steroidal anti-inflammatory drug.

52. A method for making a compound that modulates the activity of a COX-1 variant, the method comprising:
    a) carrying out the method of claim 44 to identify a compound that modulates the activity of COX-1 variant; and
    b) manufacturing the compound.

53. An article of manufacture comprising packaging material and, contained within the packaging material, a compound that modulates the activity of COX-1 variant, wherein the packaging material comprises a label or package insert indicating that said compound modulates the activity of a COX-1 variant and can be used for treating pain in a subject.

54. An article of manufacture comprising packaging material and, contained within the packaging material, a compound that modulates the activity of COX-1 variant, wherein the packaging material comprises a label or package insert indicating that said compound modulates the activity of a COX-1 variant and can be used for treating inflammation in a subject.

55. An article of manufacture comprising packaging material and, contained within the packaging material, a compound that preferentially modulates the activity of a COX-1 variant in comparison to COX-1 or COX-2, wherein the packaging material comprises a label or package insert indicating that said compound modulates the activity of a COX-1 variant and can be used for treating a subject having a COX-1 associated disorder.

56. An array comprising a substrate having a plurality of addresses, wherein at least one address of said plurality of addresses comprises a capture probe that can specifically bind a COX-1 variant nucleic acid, or a fragment of said COX-1 variant nucleic acid.

57. The array of claim 56, wherein the COX-1 variant is selected from the group consisting of COX-3, PCOX-1a), hCOX-3(cs1), hCOX-3(cs2) and hCOX-3(cs3).

58. The array of claim 56, wherein the COX-1 variant nucleic acid encodes an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

59. The array of claim 56, wherein the COX-1 variant nucleic acid is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 an SEQ ID NO:13.

60. An array comprising a substrate having a plurality of addresses, wherein at least one address of said plurality of addresses comprises a capture probe that can specifically bind intron 1 of a COX-1 variant nucleic acid.

61. The array of claim 60, wherein the COX-1 variant is selected from the group consisting of COX-3, PCOX-1a), hCOX-3(cs1), hCOX-3(cs2) and hCOX-3(cs3).

62. The array of claim 60, wherein the COX-1 variant nucleic acid encodes an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

63. The array of claim 60, wherein the COX-1 variant nucleic acid is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 an SEQ ID NO:13.

64. A method of choosing a therapy for a subject, the method comprising:
   a) providing a plurality of reference expression profiles, each associated with a therapy;
   b) providing a nucleic acid obtained from a subject;
   c) contacting the nucleic acid with an array of claim 56 or claim 60;
   d) detecting binding of the nucleic to each address of the plurality of addresses to thereby provide a subject expression profile; and
   e) selecting the reference profile most similar to the subject expression profile, to thereby choose a therapy for the subject.

65. A kit for evaluating a pharmaceutical composition, the kit comprising:

a) an array of claim 56 or claim 60; and

b) a computer-readable medium having a plurality of expression profiles, wherein each profile of the plurality has a plurality of values, each value representing the expression of a COX-1 variant nucleic acid detected by the array.

66. A method of selecting a therapy for a subject, the method comprising:

a) obtaining a subject sample from a caregiver;

b) obtaining a nucleic acid from the subject sample;

c) identifying a subject expression profile from the nucleic acid;

d) selecting from a plurality of reference profiles a matching reference profile most similar to the subject expression profile, wherein the reference profiles and the subject expression profile have a plurality of values, each value representing the expression level of a COX-1 variant;

e) wherein each reference profile of the plurality of reference profiles is associated with a therapy; and

f) transmitting a descriptor of the therapy associated with the matching reference profile to the caregiver, thereby selecting a therapy for said subject.

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A          1      8                                   Glycosylation site
                                 ** *      *              ** *      *                                                                98
Human    ---MLARALL LCAVLALSHT ANPCCSHPCQ NRGVCMSVGF DQYKCDCTRT GFYGENCSTP EFLTRIKLFL KPTPNTVHYI LTHFKGFWNV VNNIPFLRNA IMSYVLTSRS
Ovine    ---MLARALL LCAAV-VCGA ANPCCSHPCQ NRGVCMSVGF DQYKCDCTRT GFYGENCTTP EFLTRIKLLL KPTPDTVHYI LTHFKGVWNI VNKISFLRNM IMRYVLTSRS
Canine   ---MLARALV LCAALAVVRA ANPCCSHPCQ NQGICMSTGF DQYKCDCTRT GFYGENCSTP EFLTRIKLYL KPTPNTVHYI LTHFKGVWNI VNNIPFLRNT IMKYVLTSRS
Bovine   ---MLARALL LCAAVALSGA ANPCCSHPCQ NRGVCMSVGF DQYKCDCTRT GFYGENCTTP EFLTRIKLLL KPTPNTVHYI LTHFKGVWNI VNKISFLRNM IMRYVLTSRS
Equine   ---MLARALL LCVALALGHA ANPCCSNPCQ NRGVCMSVGF DQYQCDCTRT GFYGENCSTP EFLTRIKLFL KPTPNTVHYI LTHFKGVWNI VNSFPFLRNA VMKYVLVSRS
Rabbit   ---MLARALL LCAAVALSHA ANPCCSNPCQ NRGVCMTMGF DQYKCDCTRT GFYGENCSTP EFLTRIKLLL KPTPDTVHYI LTHFKGVWNI VNSIPFLRNS IMKYVLTSRS
Guinea   ---MLARALL LCAALALGQA ANPCCSNPCQ NRGECLSVGF DRYKCDCTRT GYYGENCTTP EFLTRIKLLL KPTPNTVHYI LTHFKGVWNI VNNIPFLRNA IMIYVLTSRS
Murine   ---MLFRAVL LCAALGLSQA ANPCCSNPCQ NRGECMSTGF DQYKCDCTRT GFYGENCTTP EFLTRIKLLL KPTPNTVHYI LTHFKGVWNI VNNIPFLRSL IMKYVLTSRS
Rat      ---MLFRAVL LCACPGLSHA ANPCCSNPCQ NRGECMSIGF DQYKCDCTRT GFYGENCTTP RFLTRIKLPL KPTPNTVHYI LTHFKGVWNI VNNIPFLRIQ SMRYVLTSRS
Mink     ---MLARAGL LCASLSPPHA ANPCCSNPCQ NQGVCMSIGF DQYMCDCSRT GFYGENCSTP EFLTRVKLLL KPTPNTVHYI LTHFKGVWNI VNKIPFLADV IMKYVLTSRS
Chicken  ---MLLPCAL LAALLAAGHA ANPCCSLPCQ NRGVCMTTGF DRYECDCTRT GYYGENCTTP EFFTWLKLIL KPTPNTVHYI LTHFKGVWNI INNISFLRDT IMRYVLTSRS
Rainbow  MNRVICIILL LAVGLYFCEG VDPCCAQPCE NRGLCNSKGF DNYECDCTRT GYYGKNCTTP EFLTWIKISL KPAPNTIHYI LTHYKGLWNV INKITFVRNA IMSYVLTPRS
Brook    MNKVVCIILL LTVGLYFCEG VDPCCAQPCE NRGLCNSKGF DNYECDCTRT GYYGKNCTTP EFLTWIKISL KPAPNTVHYI LTHYKGLWNV INKITFVRNA IMSYVLTSRS
             Signal peptide  EGF - like domain/dimerization domain (1)         Membrane-binding domain
         108                                 *                                                                     208
Human    HLIDSPPTYN ADYGYKSWEA FSNLSYYTRA LPPVPDDCPT PLGVKGKKQL PDSNEIVEKL LLRRKFIPDP QGSNMMFAFF AQHFTHQFFK TDHKRGPAFT NGLGHGVDLN
Ovine    HLIESPPTYN VHYSYKSWEA FSNLSYYTRA LPPVPDDCPT PMGVKGRKEL PDSKEVVKKV LLRRKFIPDP QGTNLMFAFF AQHFTHQFFK TDIERGPAFT KGKNHGVDLS
Canine   HLIESPPTYN VNYGYKSWEA FSNLSYYTRA LPPVPDDCPT PMGVKGKKEL PDSKEIVEKF LLRRKFIPDP QGTNMMFAFF AQHFTHQFFK TDHKRGPAFT KGLGHGVDLN
Bovine   HLIESPPTYN VHYSYKSWEA FSNLSYYTRA LPPVPDDCPT PMGVKGRKEL PDSKEVVKKV LLRRKFIPDP QGTNLMFAFF AQHFTHQFFK TDFERGPAFT KGKNHGVDLS
Equine   HLIESPPTYN AQYGYKSWES FSNLSYYTRA LPPVADGCPT PMGVKGKKEL PDSKEIVEKF LLRRKFIPDP QGTNMMFAFF AQHFTHQFFK TDPKRGPAFT KGLGHGVDLS
Rabbit   HMIDSPPTYN VHYNYKSWEA FSNLSYYTRA LPPVADDCPT PMGVKGKKEL PDSKDVVEKL LLRRKFIPDP QGTNMMFAFF AQHFTHQFFK TDLKRGPAFT KGLGHGVDLN
Guinea   HLIDSPPTYN AHYGYKSWEA FSNLSYYTRA LPPVADDCPT PMGVKGKKEL PDSNEVLEKV LLRRKFIPDP QGTNMMFAFF AQHFTHQFFK SDQKRGPAFT TGLAHGVDLS
Murine   YLIDSPPTYN VHYGYKSWEA FSNLSYYTRA LPPVRDDCPT PMGVKGNKEL PDSKEVLEKV LLREFIPDP QGSNMMFAFF AQHFTHQFFK TDHKRGPGFT RGLGHGVDLN
Rat      HLIDSPPTYN VHYGYKSWEA FSNLSYYTRA LPPVADDCPT PMGVKGNKEL PDSKEVLEKV LLRRKFIPDP QGTNMMFAFF AQHFTHQFFK TDQKRGPGFT RGLGHGVDLN
Mink     HCIEPPPTYN VHYAYKSWEA FSNLSYYTRA LPPVADDCPT PMGVKGKKEL PDSKEIVEKF LLRRKFIPDP QGTNMMFAFF AQHFTHQFFK TDHKRGPGFT KGLGHGVDLS
Chicken  HLIDSPPTYN SDYSYKSWEA YSNLSYYTRS LPPVGHDCPT PMGVKGKKEL PDSKLIVEKF LLRRKFIPDP QGTNVMFTFF AQHFTHQFFK TDQKKGPGFT KAYGHGVDLN
Rainbow  HLVDSPPTYN ADYGYKSWEA YSNLFYYTRT LPPLPKDCPT PMGTAGRAVL PDVKLVVEKV LLKRFIPDP QGSNLMFAFF AQHFTHQFFK SDFMKGPAFT KALGHGVDLN
Brook    HLVDSPPTYN ADYGYKSWEA YSNLSYYTRT LPPLPKDCPT PMGTAGRAVL PDVKLVVEKV LLKRFIPDP QGSNLMFAFF AQHFTHQFFK SDLKKGPAFT KALGHGVDLN
         Dimerization domain (2)  Glycosylation site                                                 Distal histidine
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FIG. 1A-1

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         218                                                                                                                  318
Human    HIYGETLARQ RKLRLFKDGK MKYQIIDGEM YPPTVKDTQA EMIYPPQVPE HLRFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWGDEQLFQT SRLILIGETI
Ovine    HVYGESLERQ HNRRLFKDGK MKYQMINGEM YPPTVKDTQV EMIYPPHIPE HLKFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWGDEQLFQT SRLILIGETI
Canine   HVYGETLDRQ HKLRLFKDGK MKYQVIDGEV YPPTVKDTQV EMIYPPHVPE HLQFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWDDERLFQT SRLILIGETI
Bovine   HIYGESLERQ HKLRLFKDGK MKYQMINGEM YPPTVKDTQV EMIYPPHVPE HLKFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWGDEQLFQT SRLILIGETI
Equine   HIYGETLDRQ HKLRLFKDGK MKYQIINGEV YPPTVKDTQV EMIYPPHIPE HLRFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWDDERLFQT SRLILIGETI
Rabbit   HIYGETLDRQ HKLRLFKDGK MKYQVIDGEV YPPTVKDTQV EMIYPPHIPA HLQFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWDDEQLFQT SRLILIGETI
Guinea   HIYGETLDRQ HKLRLFKDGK MKYQIIDGEM YPPTVKETQV EMMYPPYIPE HARFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWDDERLFQT SRLILIGETI
Murine   HIYGETLDRQ HKLRLFKDGK LKYQVIGGEV YPPTVKDTQV EMIYPPHIPE NLQFAVGQEV FGLVPGLMMY ATIWLREHNR VCDILKQEHP EWGDEQLFQT SRLILIGETI
Rat      HVYGETLDRQ HKLRLFQDGK LKYQVIGGEV YPPTVKDTQV DMIYPPHVPE HLRFAVGQEV FGLVPGLMMY ATIWLREHNR VCDILKQEHP EWDDERLFQT SRLILIGETI
Mink     HVYGETLDRQ HKLRLFKDGK MKYQVIDGEV YPPTVKDTQV EMIYPPHVPE HLRFAVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEQG EWDDERLFRR SRLILIGETI
Chicken  HIYGETLERQ LKLRLRKDGK LKYQMIDGEM YPPTVKDTQA EMIYPPHVPE HLQFSVGQEV FGLVPGLMMY ATIWLREHNR VCDVLKQEHP EWDDEQLFQT TRLILIGETI
Rainbow  HVYGDTLERQ HKLRLFKDGK LKYRVLNGEV YPPLVREVGA EMHYPPQVPE EHRFAVGHEH FGLVPGLMMY ATIWLREHNR VCDVLRQEHP EWDDERIFQT TRLILIGETI
Brook    HVYGDSLERQ HKLRLFKDGK LKYQVLNGEV YPPLVREVGA EMHYPPQVPE EHRFAVGHEM FGLVPGLMMY ATIWLREHNR VCDVLRQEHP EWDDERIFQT TRLILIGETI

                                                          Active site tyrosite
         328                                                                                                                  428
Human    KIVIEDYVQH LSGYHFKLKF DPELLFNKQF QYQNRIAAEF NTLYHWHPLL PDTFQIHDQK YNYQQFIYNN SILLEHGITQ FVESFTRQIA GRVAGGRNVP PAVQKVSQAS
Ovine    KIVIEDYVQH LSGYHFKLKF DPELLFNQQF QYQNRIAAEF NTLYHWHPLL PDVFQIDGQE YNYQQFIYNN SVLLEHGVTQ FVESFTRQIA GRVAGRRNLP AAVEKVSKAS
Canine   KIVIEDYVQH LSGYHFKLKF DPELLFNQQF QYQNRIAAEF NTLYHWHPLL PDTLQIDDQE YNFQQFIYNN SILLEHGLTQ FVESFSRQIA GRVAGGRNVP AAVQQVAKAS
Bovine   KIVIEDYVQH LSGYHFKLKF DPELLFNQQF QYQNRIAAEF NTLYHWHPLL PDVFQIDGQE YNYQQFIYNN SVLLEHGLTQ FVESFTRQRA GRVAGGRNLP VAVEKVSKAS
Equine   KIVIEDYVQH LSGYHFKLKF DPELLFNQQF QYQNRIAAEF NTLYHWHPLL PDTFQIDDQE YNFQQFLYNN SILLEHGLTQ FVESFSRQIA GRVAGGRNVP AAAQKIAKAS
Rabbit   KIVIEDYVQH LSGYHFKLKF DPELLFNQQF QYQNRIAAEF NTLYHWHPLL PDTFQIDDQQ YNYQQFLYNN SILLEHGLTQ FVESFTRQIA GRVAGGRNVP PAVQKVAKAS
Guinea   KIVIEDYVQH LSGYHFKLKF DPELLFNQQF QYQNRIASEF NTLYHWHPLL PDTFQIDDQV YNFQQFLYNN SILVEHGLTQ FVESFTKQIA GRVAGGRNVP LAVQRVAKAS
Murine   KIVIEDYVQH LSGYHFKLKF DPELLFNQQF QYQNRIASEF NTLYHWHPLL PDTFNIEDQE YSFKQFLYNN SILLEHGLTQ FVESFTRQIA GRVAGGRNVP IAVQAVAKAS
Rat      KIVIEDYVQH LRGYHFQLKF DPDLLFNQQF QYQNRIASEF KTLYHWHPLL PDTFNIEDQE YTFKQFLYNN SILLEHGLAH FVESFTRQIA GRVAGGRNVP IAVQAVAKAS
Mink     KIVIEDYVRH LSGYHFSLKF DPELLFNQQF QYQNRIAAEF NTLYHWHPLL PDTLQIDDQE YNFQQFVYNN SILLEHGLTQ FGESFSRQIA GRVAGGRNVP AAVQQEQRAS
Chicken  KIVIEDYVQH LSGYHFKLKF DPELLFNQRF QYQNRIAAEF NTLYHWHPLL PDTFQIHNQE YTFQQFLYNN SIMLEHGLSH MVKSFSKQSA GRVAGGKNVP AAVQKVAKAS
Rainbow  KIVIEDYVQH LSGYHFQLKF DPELLFNQRF QYQNRIAAEF NTLYHWHPLM PETFSIEDRA YTYPQFVFNN SLVTEHGINN LVESFTKQIA GRVAGGRNLP PALVGVAAKA
Brook    KIVIEDYVQH LSGYHFQLKF DPELLFNQRF QYQNRIAAEF NTLYHWHPLM PDTFSIEDRA YTYPQFVFNN SLVTEHGITN LVESFTKQIA GRVAGGRNLP PALVAVAAKA
                                                           Proximal heme ligand  Glycosylation site
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FIG. 1A-2

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        438                                                                                                                          538
Human   IDQSRQMKYQ SFNEYRKRFM LKPYESFEEL TGEKEMSAEL EALYGDIDAV ELYPALLVEK PRPDAIFGET MVEVGAPFSL KGLMGNVICS PAYWKPSTFG GEVGFQIINT
Ovine   LDQSREMKYQ SFNEYRKRFL LKPYESFEEL TGEKEMAAEL EALYGDIDAM ELYPALLVEK PAPDAIFGET MVEAGAPFSL KGLMGNPICS PEYWKPSTFG GEVGFKIINT
Canine  IDQSRQMKYQ SLNEYRKRFR LKPYTSFEEL TGEKEMAAGL EALYGDIDAM ELYPALLVEK PRPDAIFGET MVEMGAPFSL KGLMGNPICS PDYWKPSTFG GEVGFKIINT
Bovine  IDQSREMKYQ SFNEYRKRFL VKPYESFEEL TGEKEMAAEL EALYGDIDAM EFYPALLVEK PRPDAIFGET MVEAGAPFSL KGLMGNPICS PEYWKPSTFG GEVGFKIINT
Equine  IDQSREMKYQ SLNEYRKRFR LTPYKSFEEL TGEKEMAAEL EALYGDIDAM ELYPALLVEK PRPDAIFGET MVELGAPFSL KGLLGNPICS PDYWKPSTFG GEVGFKIINT
Rabbit  IDQSRQMKYQ SLNEYRKRFL LKPYESFEEL TGEKEMAAEL EALYGDIDAV ELYPALLVER PRPDAIFGES MVEMGAPFSL KGLMGNPICS PNYWKPSTFG GEVGFKIVNT
Guinea  IEHSRKMKYQ SLNEYRKRFL MKPYTSFEEL TGEKEMAAGL EALYGDIDAM ELYPALLVEK PRPDAIFGET MVEMGAPFSL KGLMGNPICS PHYWKPSTFG GEVGFQIVNT
Murine  IDQSREMKYQ SLNEYRKRFS LKPYTSFEEL TGEKEMAAEL KALYSDIDVM ELYPALLVEK PRPDAIFGET MVELGAPFSL KGLMGNPICS PQYWKPSTFG GEVGFKIINT
Rat     IDQSREMKYQ SLNEYRKRFS LKPYTSFEEL TGEKEMAAEL KALYHDIDAM ELYPALLVEK PRPDAIFGET MVELGAPFSL KGLMGNPICS PQYWKPSTFG GEVGFRIINT
Mink    IDQSRQMKYQ SLNEYRKRFS VKPYASFEEL TGEKEMAGEL KALYQDIDAM ELYPALLVEK PRPDAIFGET MVEIGAPFSL KGLMGNPICS PDYWKPSHFG GEVGFKIINT
Chicken IDQSRQMRYQ SLNEYRKRFM LKPFKSFEEL TGEKEMAAEL EELYGDIDAM ELYPGLLVEK PRPGAIFGET MVEIGAPFSL KGLMGNTICS PEYWKPSTFG GKVGFEIINT
Rainbow LEHSRDMRYQ SLNAYRKRFN MRVYTSFEDL TGETELAAEL ESLYGDVDAV ELYPGLLVER PRPNAVFGET MVEMGAPYSL KGLLGNPICS PEYWMPSTFG GSVGFDILNT
Brook   LEHSRDMRYQ SLNAYRKRFN MRAYTSFEDL TGETELAAEL ESLYGDVDAV ELYPGLLVER PRPNAVFGET MVEMGAPYSL KGLLGNPICS PEYWMPSTFG GSVGFDIVNT
                                                              Glycosylation site   Active site valine   ASA-acetylated serine
        538    *     *                                           597
Human   ASIQSLICNN VKG-CPFTSF SVPDPELIKT VTINASSSRS GLDDINPTVL LKERSTEL
Ovine   ASIQSLICSN VKG-CPFTSF SVQDAHLTKT VTINASSSHS GLDDINPTVL LKERSTEL
Canine  ASIQSLICNN VKG-CPFTAF SVQDGQLTKT VTINASSSHS GLDDINPTVL LKERSTEL
Bovine  ASIQSLICSN VKG-CPFTSF SVQDTHLTKT VTINASSSHS GLDDINPTVL LKERSTEL
Equine  ASIQSLICNN VKG-CPFTAF SVQDPQLSKA VTINASASHS GLDDVNPTVL LKERSTEL
Rabbit  ASIQSLICNN VKG-CPFTSF NVPDPQLTKT VTINASASHS RLEDINPTVL LKGRSTEL
Guinea  ASIQSLICNN VKG-CPATAF NLPDPQLAKT VTINASASHS RLEDLSPTVL LKGRSTEL
Murine  ASIQSLICNN VKG-CPFTSF NVQDPQPTKT ATINASASRS RLDDINPTVL IKRRSTEL
Rat     ASIQSLICNN VKG-CPFASF NVQDPQATKT ATINASASHS RLDDINPTVL IKRRSTEL
Mink    ASIQSLICNN VKG-CPFTAF SVQDPQLTKT VTINGSSSHS GLDDINPTVL LKERSTEL
Chicken ASLQKLICNN VKG-CPFTAF HVLNPEPTE- ATINVSTSNT AMEDINPTLL LKEQSAEL
Rainbow ASLERLVCNN VKGSCPMVSF QVPD-FLRAF ESASVNTSEA HLSDMNPGVL FKERTSEL
Brook   ASLERLVCNN VKGSCPMVSF QVPD-FLRAF ESASVNTSEA HLRGMNPGVV FKERTLEL
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FIG. 1A-3

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B                                                       24                **  *     *         * *         *   74
Human   ---------- ---------- -MSR-SLLLR FL---LFLLL L---PPLPVL LADPGAPTPV NPCCYYPCQH QGICVRFGLD RYQCDCTRTG YSGPNCTIPG LWTWLRNSLR
Ovine   ---------- ---------- -MSRQSILLR FP---LLLLL L---SPSPVF SADPGAPAPV NPCCYYPCQH QGICVRFGLD RYQCDCTRTG YSGPNCTIPE IWTWLRTTLR
Canine  ---------- ---------- -MSRGSRLHR WP---LLLLL LLLLPPPPVL PAEARTPAPV NPCCYYPCQH QGICVRFGLD RYQCDCTRTG YSGPNCTIPE LWTWLRNSLR
Rabbit  ---------- ---------- -MSRSSPSLR LPVLLLLLLL LLLPPPPPVL PADPGAPAPV NPCCYFPCQH QGVCVRVALD RYQCDCTRTG YSGPNCTVPD LWTWLRSSLR
Murine  ---------- ---------- -MSRRSLSLW FP----LLLL LLLPPTPSVL LADPGVPSPV NPCCYYPCQN QGVCVRFGLD NYQCDCTRTG YSGPNCTIPE IWTWLRNSLR
Rat     ---------- ---------- -MSRRSLSLQ FP----LLLL LLLPPPPPVL LTDAGVPSPV NPCCYYPCQN QGVCVRFGLD HYQCDCTRTG YSGPNCTIPE IWTWLRSSLR
Rainbow MSAAYIIFAL LYWEDAPAEG AYAVNLTMRE CVVWVWACIL LQRLPTCRGE EVEDAST-VV NPCCYYPCQN WGVCVRFGID RYECDCTRTG FYGQNCTIPE FWTRVHQQLK
Brook   ---------- ---------- -------MRG LVVCVWACIL LQRLPTCRGE EVKDVSTNVV NPCCYYPCQN WALCVRFGID RYECDCTRTG FYGQNCTIPE FWTRIHQQLK
                                   Signal peptide                       EGF-like domain/dimerization domain (1)
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          Glycosylation site
        84                                                                                                        184
Human   PSPSFTHFLL THGRWFWEFV NATFIREMLM RLVLTVRSNL IPSPPTYNSA HDYISWESFS NVSYYTRILP SVPKDCPTPM GTKGKKQLPD AQLLARRFLL RRKFIPDPQG
Ovine   PSPSFIHFML THGRWLWDFV NATFIRDTLM RLVLTVRSNL IPSPPTYNIA HDYISWESFS NVSYYTRILP SVPRDCPTPM GTKGKKQLPD AEFLSRRFLL RRKFIPDPQG
Canine  PSPSFLHFLL THGRWFWEFI NATFIRDMLM RLVLTARSNL IPSPPTYNIA HDYISWESFS NVSYYTRVLP SVPQDCPTPM GTKGKKQLPD AQLLGRRFLL RRKFIPDPQG
Rabbit  PSPTFVHYLL THVRWFWEFV NATFIRDTLM RLVLTVRSNL IPSPPTYNLA YDYISWEAFS NVSYYTRVLP SVPKDCPTPM GTKGKKQLPD AQVLAHRFLL RRTFIPDPQG
Murine  PSPSFTHFLL THGYWLWEFV NATFIREVLM RLVLTVRSNL IPSPPTYNSA HDYISWESFS NVSYYTRILP SVPKDCPTPM GTKGKKQLPD VQLLAQQLLL RREFIPAPQG
Rat     PSPSFTHFLL THGYWIWEFV NATFIREVLM RLVITVRSNL IPSPPTYNTA HDYISWESFS NVSYYTRILP SVPKDCPTPM GTKGKKQLPD IHLLAQRLLL RREFIPGPQG
Rainbow PSPDVVHYIL THFHWLWNLI NRTFMRDWLM RVVLTVRSNL IPSPPTFNSK YGYLSWESYS NVSYYTRILP PVPEDCPTPM GTKGKSVLPD PKLVVEKFLL RRQFRRDPRG
Brook   PSPDVVHYIL THFHWLWNLI NRTFMRDWLM RMVLTVRSNL IPSPPTFNSK YGYLSWESYS NVSYYTRILP PVPEDCPTPM GTKGKSVLPD PKLVVEKFLL RRQFRPDPKG
           Membrane-binding domain         Dimerization domain (2)   Glycosylation site
```

FIG. 1B-1

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        194                                                                                                             294
Human   TNLMFAFFAQ HFTHQFFKTS GKMGPGFTKA LGHGVDLGHI YGDNLERQYQ LRLFKDGKLK YQVLDGEMYP PSVEEAPVLM HYPRGIPPQS QMAVGQEVFG LLPGLMLYAT
Ovine   TNLMFAFFAQ HFTHQFFKTS GKMGPGFTKA LGHGVDLGHI YGDNLERQYQ LRLFKDGKLK YQMLNGEVYP PSVEEAPVLM HYPRGIPPQS QMAVGQEVFG LLPGLMLYAT
Canine  TNLMFAFFAQ HFTHQFFKTS GKMGPGFTKA LGHGVDLGHI YGDNLDRQYQ LRLFKDGKLK YQVLDGEMYP PSVEEAPVLM HYPRGILPQS QMAVGQEVFG LLPGLMLYAT
Rabbit  TNLMFAFFAQ HFTHQFFKTS GKMGPGFTKA LGHGVDLGHI YGDSLERQYH LRLFKDGKLK YQVLDGEVYP PSVEEAPVLM HYPRGVPPRS QMAVGQEVFG LLPGLMLYAT
Murine  TNIMFAFFAQ HFTHQFFKTS GKMGPGFTKA LGHGVDLGHI YGDNLERQYH LRLFKDGKLK YQVLDGEVYP PSVEQASVLM RYPPGVPPER QMAVGQEVFG LLPGLMLFST
Rat     TNVLFAFFAQ HFTHQFFKTS GKMGPGFTKA LGHGVDLGHI YGDSLERQYH LRLFKDGKLK YQVLDGEVYP PSVEQASVLM RYPPGVPPEK QMAVGQEVFG LLPGLMLFST
Rainbow TNLMFAFFAQ HFTHQFFKTR NSMGLGFTRA LGHGVDAGNV YGDNLVRQLN LRLLKDGKMK YQVVKGEVYP PTVAEAAVNM RYPQETPVGQ RMAIGQEVFG LLPGLTMYAT
Brook   TNLMFAFFAQ HFTHQFFKTR NSMGLGFTSA LGHGVDAGNV YGDNLVRQLN LRLLKDGKMK YQVVKGEVYP PTVAEAPVNM RYPQGTPVGQ RMAIGQEVFG LLPGLTMYAT
                 Distal histidine

                                                                                                       Glycosylation site
        304                                                                                                             404
Human   LWLREHNRVC DLLKAEHPTW GDEQLFQTTR LILIGETIKI VIEEYVQQLS GYFLQLKFDP ELLFGVQFQY RNRIAMEFNH LYHWHPLMPD SFKVGSQEYS YEQFLFNTSM
Ovine   LWLREHNRVC DLLKAEHPTW GDEQLFQTAR LILIGETIKI VIEEYVQQLS GYFLQLKFDP ELLFGVQFQY RNRIAMEFNQ LYHWHPLMPD SFRVGPQDYS YEQFLFNTSM
Canine  LWLREHNRVC DLLKAEHPTW GDEQLFQTAR LILIGETIKI VIEEYVQQLS GYFLQLKFDP ELLFSVQFQY RNRIAMEFNQ LYHWHPLMPD SFWVGSQEYS YEQFLFNTSM
Rabbit  LWLREHNRVC DLLKAEHPTW DDEQLFQTTR LILIGETIKI VIEEYVQQLS GYFLQLKFDP EMLFSVQFQY RNRIAMEFNH LYHWHPLMPD SFQVGSQEYS YEQFLFNTSM
Murine  IWLREHNRVC DLLKEEHPTW DDEQLFQTTR LILIGETIKI VIEEYVQHLS GYFLQLKFDP ELLFRAQFQY RNRIAMEFNH LYHWHPLMPN SFQVGSQEYS YEQFLFNTSM
Rat     IWLREHNRVC DLLKEEHPTW DDEQLFQTTR LILIGETIKI IIEEYVQHLS GYFLQLKFDP ELLFRAQFQY RNRIALEFNH LYHWHPLMPD SFQVGSQEYS YEQFLFNTSM
Rainbow LWLREHNRVC DILKAEHPTW GDEQLFQTAR LIVIGETIRI VIEEYVQHLS GYLLDLKFDP VLLFKSTFQY RNRIAVEFKH LYHWHPLMPD SFHIDGDEVP YSQFIFNTSI
Brook   LWLREHNRVC DILKAEHPTW GDEQLFQTAR LIVIGETIRI VIEEYVQHLS GYLLDLKFDP VLLFKSTFQY RNRIAVEFNQ LYHWHPLMPD SFHIDGDVVS YSQFIFNTSI
                                                                                     Active site tyrosine  Proximal heme ligand
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FIG. 1B-2

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        414                                                                                                                       514
Human   LVDYGVEALV DAFSRQIAGR IGGGRNMDHH ILHVAVDVIR ESREMRLQPF NEYRKRFGMK PYTSFQELVG EKEMAAELEE LYGDIDALEF YPGLLLEKCH PNSIFGESMI
Ovine   LVDYGVEALV DAFSRQPAGR IGGGRNIDHH ILHVAVDVIK ESRVLRLQPF NEYRKRFGMK PYTSFQELTG EKEMAAELEE LYGDIDALEF YPGLLLEKCH PNSIFGESMI
Canine  LTHYGIEALV DAFSRQSAGR IGGGRNIDHH VLHVAVETIK ESRELRLQPF NEYRKRFGMR PYMSFQELTG EKEMAAELEE LYGDIDALEF YPGLLLEKCH PNSIFGESMI
Rabbit  LVDYGVEALV DAFSRQSAGR IGGGRNIDHH VLHVAVEVIK ESREMRLQPF NEYRKRFGLK PYASFQELTG ETEMAAELEE LYGDIDALEF YPGLLLEKCQ PNSIFGESMI
Murine  LVDYGVEALV DAFSRQRAGR IGGGRNFDYH VLHVAVDVIR ESREMRLQPF NEYRKRFGLK PYTSFQELTG EKEMAAELEE LYGDIDALEF YPGLLLEKCQ PNSIFGESMI
Rat     LVDYGVEALV DAFSRQRAGR IGGGRNFDYH VLHVAEDVIK ESREMRLQSF NEYRKRFGLK PYTSFQEFTG EKEMAAELEE LYGDIDALEF YPGLMLEKCQ PNSLFGESMI
Rainbow VTHYGVEKLV DAFSRQCAGQ IGGGRNIHPV VTNVAEGVIE ESRTLRLQPF NEYRKRFNLK PYTSFSDFTG EEEMARELEE LYGDIDALEF YPAIMLEKTR PNAIFGESMV
Brook   VTHYGVEKLV DAFSRQYAGQ IGGGRNIHPV VTKVAEGVIE ESRTLRLQPF NEYRKRFNLK PYTSFSDFTG EEEMARELEE LYGDIDALEF YPALMLEKTR PNAIFGESMV
                                                                                                            Active site isoleucine

        524    ASA-acetylated serine
                                                     *         *                    594
Human   EIGAPFSLKG LLGNPICSPE YWKPSTFGGE VGFNIVKTAT LKKLVCLNTK TCPYVSFRVP DASQDDGPAV ERPSTEL
Ovine   EMGAPFSLKG LLGNPICSPE YWKASTFGGE VGFNLVKTAT LKKLVCLNTK TCPYVSFHVP DPRQEDRPGV ERPPTEL
Canine  EIGAPFSLKG LLGNPICSPE YWKPSTFGGE MGFNMVKTAT LKKLVCLNTK TCPYVSFRVP DPHQDGGPGV ERPSTEL
Rabbit  EIGAPFSLKG LLGNPICSPE YWKPSTFGGE VGSNLIKTAT LKKLVCLNTK TCPYVSFRVP RSSGDDGPAA ERRSTEL
Murine  EMGAPFSLKG LLGNPICSPE YWKPSTFGGD VGFNLVNTAS LKKLVCLNTK TCPYVSFRVP DYPGDDGSLV VRRSTEL
Rat     EMGAPFSLKG LLGNPICSPE YWKPSTFGGD VGFNIVNTAS LKKLVCLNTK TCPYVSFRVP DYPGDDGSVF VRPSTEL
Rainbow EMGAPFSLKG LLGNPICSPE YWKPSTFGGQ TGFDIVNSAS LERLVCLNTN WCPYVAFNVP PAGQEEPP-- RKQSTEL
Brook   EMGAPFSLKG LLGNPICSPE YWKPSTFGGQ TGFDIVNSAS LERLVCLNTN WCPYVAFNVP PAGQEPPP-- RKQSTEL
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FIG. 1B-3

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COX-1    MSR.S.SLRF P..LLLLLLL PPPPVL.ADP GVP.PVNPCC YYPCQHQGVC VRFGLDRYQC DCTRTGYSGP NCTIPE.WTW LR.SLRPSPS F.HFLLTHGR WLWEFVN-AT
COX-2    ---------M LARALLLCAA LAL..A---- -----ANPCC S.PCQNRGVC MS.GFDQYKC DCTRTGFYGE NCTTPEFLTR IKL.LKPTPN TVHYILTHFK GVWNIVN.IP
Plexaura ---------- --MKAFLHIV VLIIFSGLFW HEVESVNPCC SFPCENGAVC VDDG-DTYTC DCTRTGYYGV NCEKPSWSTW LKSFIKPSEQ TKHFMLTHFG WFWWIVNNVQ
Gersemia ---------- -MVAKFVVFL GLQLILCSV- -VCEAVNPCC SFPCESGAVC VEDG-DKYTC DCTRTGHYGV NCEKPNWSTW FKALIAPSEE TKHFILTHFK WFWWIVNNVP
              Signal peptide                 EGF-like domain/dimerization domain (1)         Membrane-binding domain

                                                               *
COX-1    FIRD.LMRLV LTVRSNLIPS PPTYNSAHDY ISWESFSNVS YYTRILPSVP KDCPTPMGTK GKKQLPDAQL LA.RFLLRR. FIPDPQGTNL MFAFFAQHFT HQFFKTSGKM
COX-2    FLRN.IM.YV LTSRSHLIDS PPTYNVHYGY KSWEAFSNLS YYTRALPPV. DDCPTPMGVK GKKELPDSKE VVEKVLLRRK FIPDPQGTNM MFAFFAQHFT HQFFKTD.KR
Plexaura FIRDPIMRAA YFSRTDFIPV PHVYTSYHEY ATMEAHYNRT HFARTLPPVP KNCPTPFGVS GKKILPPAEE VANKFLKRRE FIADHRNTSW LFMFFAQHFT HQFFKTVHH-
Gersemia FIRNTVMKAA YFSRTDFVPV PHAYTSYHDY ATMEAHYNRS YFARTLPPVP KNCPTPFGVA GKKELPPAEE VANKFLKRGK FKTDHTSTSW LFMFFAQHFT HEFFKTIYH-
                   Dimerization domain (2)  Glycosylation site                               Distal histidine

COX-1    GPGFTKALGH GVDLGHIYGD NLERQY.LRL FKDGKLKYQV LDGEVYPPSV EEAP-VLMHY PRG.PP.SQM AVGQEVFGLL PGLMLYATLW LREHNRVCDL LKAEHPTWGD
COX-2    GPAFTKGLGH GVDLNHIYGE TLDRQHKLRL FKDGKMKYQV I.GEVYPPTV KDTQ-VEMIY PPHVPEHLRF AVGQEVFGLV PGLMMYATIW LREHNRVCDV LKQEHPEWDD
Plexaura SPAFSWG-NH GVDVSHIYGQ GVERENKLRA FKDGKLKSQM INGEEYPPYL KDVDGLKMQY LENTAEEQKF ALGHPFFSML PGLFMFATLW LREHNRVCMI LRKEHPHWED
Gersemia SPAFTWG-NH GVDVSHIYGQ DMERQNKLRS FEDGKLKSQT INGEEWPPYL KDVDNVTMQY PPNTPEDQKF ALGHPFYSML PGLFMYASIW LREHNRVCTI LRKEHPHWVD

                                                                                 Glycosylation site
COX-1    EQLFQTARLI LIGETIKIVI EEYVQHLSGY FLQLKFDPEL LF.AQFQYRN RIAMEFNHLY HWHPLMPDSF .VGSQEYSYE QFLFNTSMLV DYGVEALVDA FSRQ.AGRIG
COX-2    ERLFQTSRLI LIGETIKIVI EDYVQHLSGY HFKLKFDPEL LFNQQFQYQN RIAAEFNTLY HWHPLLPDTF QIDDQEYNFQ QF.YNNSILL EHGLTQFVES FTRQIAGRVA
Plexaura ERIYQTAKLI ITGETIKIVI EDYINHLANY NMKLRYDPQL VFSRNYDYDN RIHLEFNHLY HWHPFSPDQF NISGTTYTIN DFMYHPEIVV KHGMSSFVNA MSSGLCGKMS
Gersemia ERLYQTGKLI ITGELIKIVI EDYVNHLANY NLKLTYNPEL VFDHGYDYDN RIHVEFNHMY HWHPFSPDEY NISGSTYSIQ DFMYHPEIVV KHGMSSFVDS MSKGLCGQMS
                                                Active site tyrosine  Proximal heme ligand
```

FIG. 1C-1

```
COX-1    GGRNIDHHVL HVAVDVIKES RELRLQPFNE YRKRFGLKPY TSFQELTGEK EMAAELEELY GDIDALEFYP GLLLEKC.PN SIFGESMIEM GAPFSLKGLL GNPICSPEYW
COX-2    GGRNVP.AVQ .VAKASIDQS R.MKYQSLNE YRKRF.LKPY .SFEELTGEK EMAAELEALY GDIDAMELYP ALLVEKPRPD AIFGETMVE. GAPFSLKGLM GNPICSP.YW
Plexaura H-HNHGQYTL DVAVEVIKYQ RKLRMQSFNN YRRHFGLPAY KSFEEMTGDP KLAAELKEVY GDVNAVDFYV GFFLEKSLPT SPFGITMIAS GAPYSLRGLL SNPVSSPTYW
Gersemia H-HNHGAYTL DVAVEVIKHQ RELRMQSFNN YRKHFGLEPY KSFEELTGDP KMSAELQEVY GDVNAVDLYV GFFLEKGLTT SPFGITMIAF GAPYSLRGLL SNPVSSPTYW
                                                                                          Active site valine/isoleucine     ASA-acetylated serine

                                 *         *
COX-1    KPSTFGGEVG FNIVKTASLK KLVCLNTKT- CPYVSFRVPD ..QDDGP..E ---------- ---------- RPSTEL
COX-2    KPSTFGGEVG FKIINTASIQ SLICNNVKG. CPFTSF.VQD PQLT.KTVTI NAS.SHS.LD DINPTVLLKE R-STEL
Plexaura KPSTFGGEVG FDIVKTASVD KLFCRNIAGD CPLVTFTVPD EIAREARRNL AANI------ ---------- --KDEL
Gersemia KPSTFGGDVG FDMVKTASLE KLFCQNIAGE CPLVTFTVPD DIARETRKVL EA-------- ---------- --RDEL
```

FIG. 1C-2

```
COX-1       MSR.S.SLRF P..LLLLLLL PPPPVL.ADP GVP.PVNPCC YYPCQNQGVC VRFGLDRYQC DCTRTGYSGP NCTIPE.WTW LR.SLRPSPS F.HFLLTHGR WLWEFVN-AT
COX-2       MLARALLLCA ALAL..A--- ---------- -----ANPCC S.PCQNRGVC MS.GFDQYKC DCTRTGFYGE NCTTPEFLTR IKL.LKPTPN TVHYILTHFK GVWNIVN.IP
Rice        ---------- ---------M GSGLFKPRVH PDLRDVFSKM SF-FDKIGFL FIHAFDKRNL WHKVPVPIGL LYLNTRRTLL EKYNLLAVGR SSHGALFDPK EFLYRTEDGK
A. Thaliana ---------- ---MKVITSL ISSILLKFIH KDFHEIYARM SL-LDRFLLL IVHGVDKMVP WHKLPVFLGL TYLEVRRHLH QQYNLLNVGQ TPTGIRFDPA NYPYRTADGK
Tobacco     ---------M SLVMASLKNL LLSPLRGFIH KDFHDIFERM TL-LDKLFFL IVHFVDKLNL WHRLPVFLGL LYLGARRHLH QEYNLINVGK TPVGVRSNPA DHPYRTADGK
                       Signal peptide          EGF-like domain/dimerization domain (1)        Membrane-binding domain

COX-1       FIRD.LMRLV LTVRSNLIPS PPTYNSAHDY ISWESF--SN VSYYTRILPS VPKDCPTPMG TKGKKQLPDA QLLA.RFLLR R.FIPDPQGT NLMFAFFAQH FTHQFFKTSG
COX-2       FLRN.IM.YV LTSRSHLIDS PPTYNVHYGY KSWEAF--SN LSYYTRALPP V.DDCPTPMG VKGKKELPDS KEVVEKVLLR RKFIPDPQGT NMMFAFFAQH FTHQFFKTD.
Rice        YNDPHNAEAG KPKHLFWGET WSRLINRNEL MSPDPFVVAT KLLARREYKD TGKQFNILAA AWIQFMVHDW MDHMEDTGQI GITAPKEVAN ECPLKSFKFH PTKELPTNSD
A. Thaliana FNDPFNEGVG S-QNSFFGRN CPPVDQKSKL RRPDPMVVAT KLLGRKKFID TGKQFNMIAA SWIQFMIHDW IDHLEDTHQI ELVAPKEVAS KCPLSSFRFL KTKEVPTGFF
Tobacco     YNDPFNEGAG S-ELSFFGRN MLPVDQHNQL KKPDPMVVAT KLLARRNVVD TGKQFNMIAA SWIQFMIHDW IDHLEDTKQI ELRAPEEVAS QCPLKSFKFF KTKEIPTGFY
                          Dimerization domain (2)                                                          Distal histidine

COX-1       KMGPGFTKAL GHGVDLGHIY GDNLERQY.L RLFKDGKLKY QVLDGEVYPP SVEEAPVLMR YPRG.PP.SQ MAVGQEVFGL LPGLMLYATL WLREHNRVCD LLKAEHPTWG
COX-2       KRGPAFTKGL GHGVDLNHIY GETLDRQHKL RLFKDGKMKY QVI.GEVYPP TVKDTQVEMI YPPHVPEHLR FAVGQEVFGL VPGLMMYATI WLREHNRVCD VLKQEHPEWD
Rice        GIKIGHYNIR TAWWDGSAVY GNNEERAEKL RTYVDGKLVI GDD-GLL--- ---------- ----LHKENG VALSGDIRNS WAGVSILQAL FVKEHNAVCD AIKEEHPNLS
A. Thaliana EIKTGSQNIR TPWWDSSVIY GSNSKTLDRV RTYKDGKLKI SEETGLL--- ---------- ----LHDEDG LAISGDIRNS WAGVSALQAL FIKEHNAVCD ALKDEDDDLE
Tobacco     EIKTGHLNRR TPWWDGSAIY GSNAEVLKKV RTFKYGKLKL SAD-GLL--- ---------- ----EIDENG KIISGDVRNT WAGLSALQAL FVQEHNSVCD VLKKEYPELE
                                                                                                           "REHN" sequence

COX-1       DEQLFQTARL ILIGETIKIV IEEYVQHLS- ---------- ------GYFL QLKFDP---- ------ELLF .AQFQYRNRI AMEFNQLYHW HPLMPDSF.V GS--------
COX-2       DERLFQTSRL ILIGETIKIV IEDYVQHLS- ---------- ------GYHF KLKFDP---- ------ELLF NQQFQYQNRI AAEFNTLYHW HPLLPDTFQI DD--------
Rice        DEELYRYAKL VTSAVIAKVH TIDWTVELLK TKTMRAAMRA NWYGLLGKKI KDTFGHIGGP ILGGLVGLKK PNNHGVPYSL TEEFTSVYRM HSLIPSTLKL RDPTGQPDAN
A. Thaliana DEDLYRYARL VTSAVVAKIH TIDWTVQLLK TDTLLAGMRA NWYGLLGKKF KDSFGHAGSS ILGGVVGMKK PQNHGVPYSL TEDFTSVYRM HSLLPDQLHI LDIDDVPGTN
Tobacco     DEDLYRHARL VTSAVIAKVH TIDWTVELLK TDTLLAGMRA NWYGLLGKKF KDTFGHVGGS ILGGFVGMKK PENYGVPYSL TEEFTSVYRM HQLLPDNLQL RNIDATPGPN
                                                                                           Active site tyrosine   Proximal heme ligand
```

FIG. 2-1

```
COX-1       ------QEYS YEQFL--FNT SMLVDYGVEA LVDAFSRQ.A GRIG------ ---------- GGRNIDHHVL HVAVDVIKES REMRLQPFNE YRKRFGLKPY TSFQELTGEK
COX-2       ------QEYN FQQF.--YNN SILLEHGLTQ FVESFTRQIA GRVA------ ---------- GGRNVP.AVQ .VAKASIDQS R.MKYQSLNE YRKRF.LKPY .SFEELTGEK
Rice        NSPPCLEDID IGEMIGLKGE EQLSKIGFEK QALSMGYQAC GALELWNYPS FFRNLIPQNL DGTNRSDRI- DLAALEVYRD RERSVPRYNE FRRRLFLIPI KSWEDLTSDK
A. Thaliana KSLPLIQEIS MRDLIGRKGE ETMSHIGFTK LMVSMGHQAS GALELMNYPM WLRDIVPHDP NGQARPDHV- DLAALEIYRD RERSVPRYNE FRRSMFMIPI TKWEDLTEDE
Tobacco     KSLPLTNEIP MEDLIGSKGE ENLARIGFTK QMVSMGHQAC GALELWNYPM WMRDLIPQDV DGTDRPDHV- DLAALEIYRD RERSVARYNE FRRGMLQIPI SKWEDLTDDE

COX-1       EMAAELEELY G-DIDALEFY PGLLLEKC.P NSIFGESMIE MGAPFSLKGL LGNPICSPEY WKPSTFGGEV GFNIVNTASL KKLVCLNTKT -CPYVSFRVP D..QDDGP..
COX-2       EMAAELEALY G-DIDAMELY PALLVEKPRP DAIFGETMVE .GAPFSLKGL MGNPICSP.Y WKPSTFGGEV GFKIINTASI QSLICNNVKG .CPFTSF.VQ DPQLT.TVTI
Rice        DAIETIRAIY GDDVEKLDLL VGLMAEKKIK GFAISETAFN IFILMASRRL EADRFFTSNF -NEETYTKKG MQWVKTTEGL RDVINRHYPE ITAKWMKSSS AFSVWDADY-
A. Thaliana EAIEVLDDVY DGDVEELDLL VGLMAEKKIK GFAISETAFY IFLIMATRRL EADRFFTSDF -NETIYTKKG LEWVNTTESL KDVIDRHYPD MTDKWMNSES AFSVWDSPPL
Tobacco     EVINTLREVY GDDVEELDLM VGMAAEKKIK GFAISETAFF IFLIMASRRL EADRFFTSNY -NEETYTKKG LEWVNTTESL KDVLDRHYPE MTEKWMNSSS AFSVWDSSPE

COX-1       ERP------- ---------- -STEL
COX-2       NAS.SHS.LD DINPTVLLKE RSTEL
Rice        ---------- ---------- -----
A. Thaliana TKNPIPLYLR IPS------- -----
Tobacco     PHNPIPLYFR VPPQ------ -----
```

FIG. 2-2

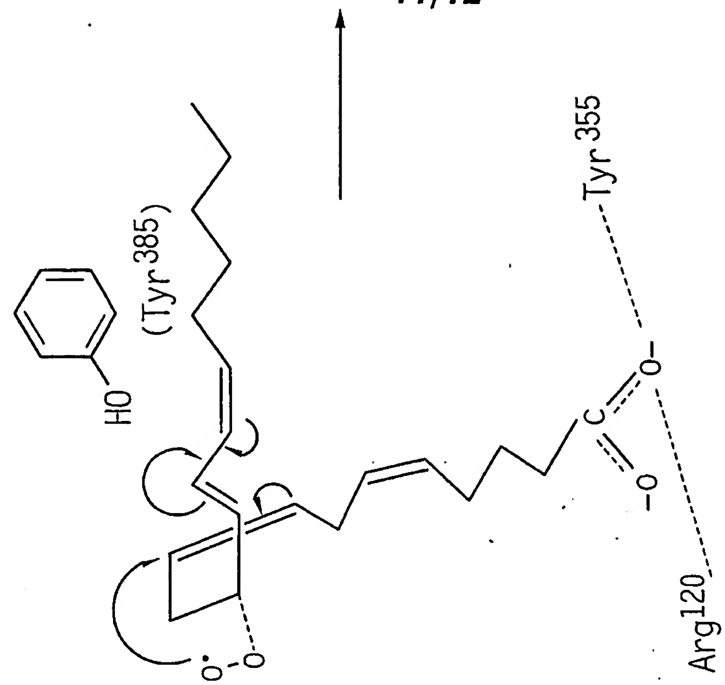
(Tyr 385)
HO
Tyr 355
Arg120

FIG. 3B

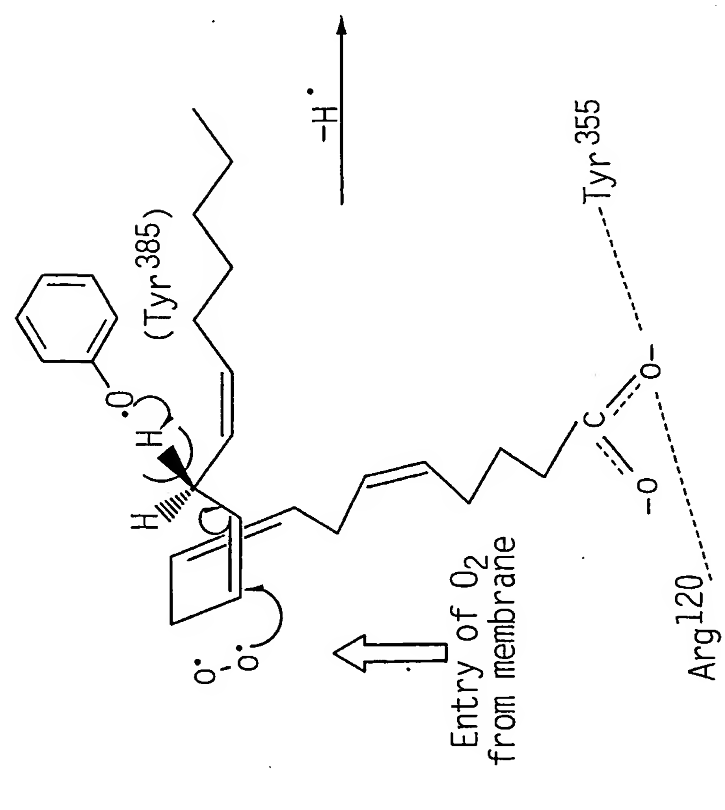
(Tyr 385)
$-H^{\bullet}$
Tyr 355
Arg120
Entry of O_2 from membrane

FIG. 3A

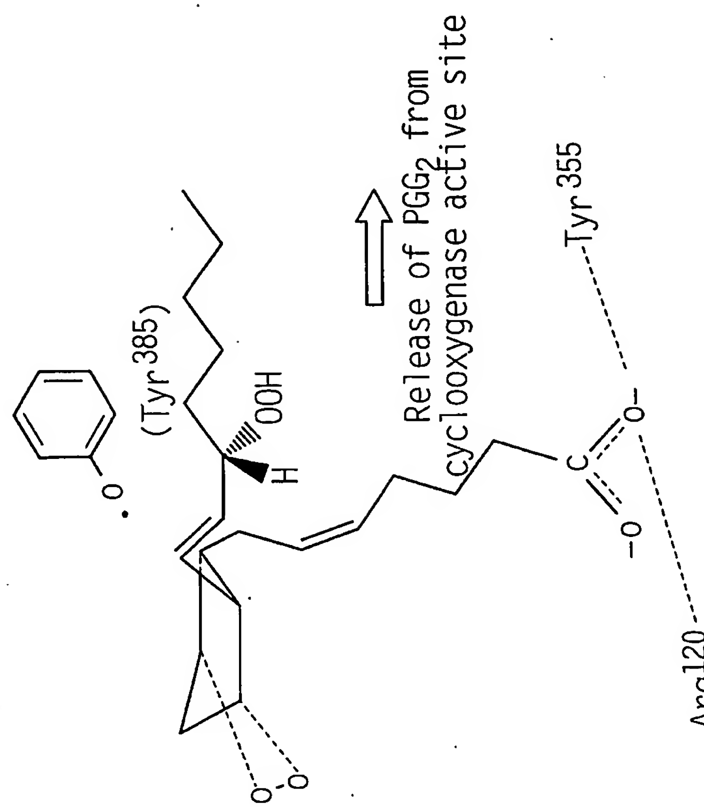
(Tyr 385)
OOH
H
Release of PGG_2 from
cyclooxygenase active site
Tyr 355
Arg120

FIG. 3D

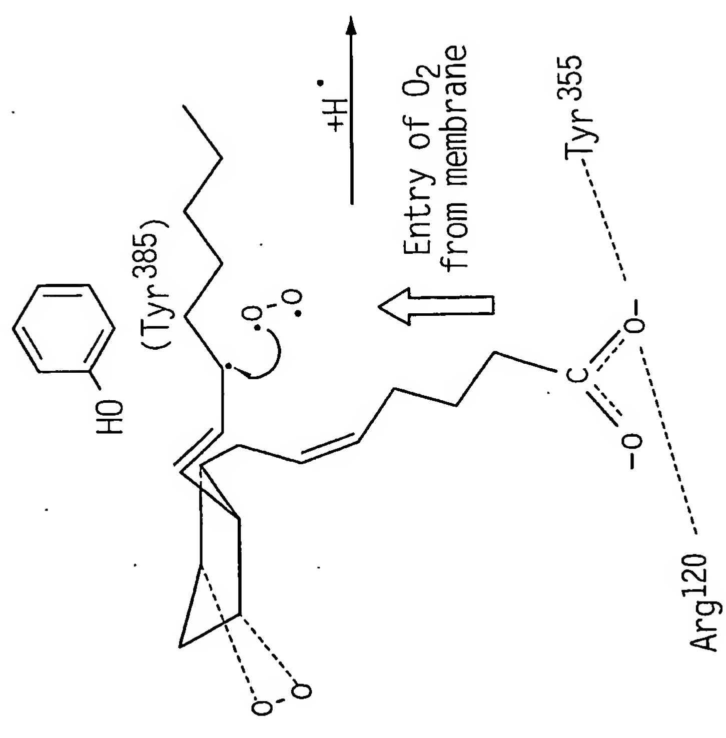
HO
(Tyr 385)
+H•
Entry of O_2
from membrane
Tyr 355
Arg120

FIG. 3C

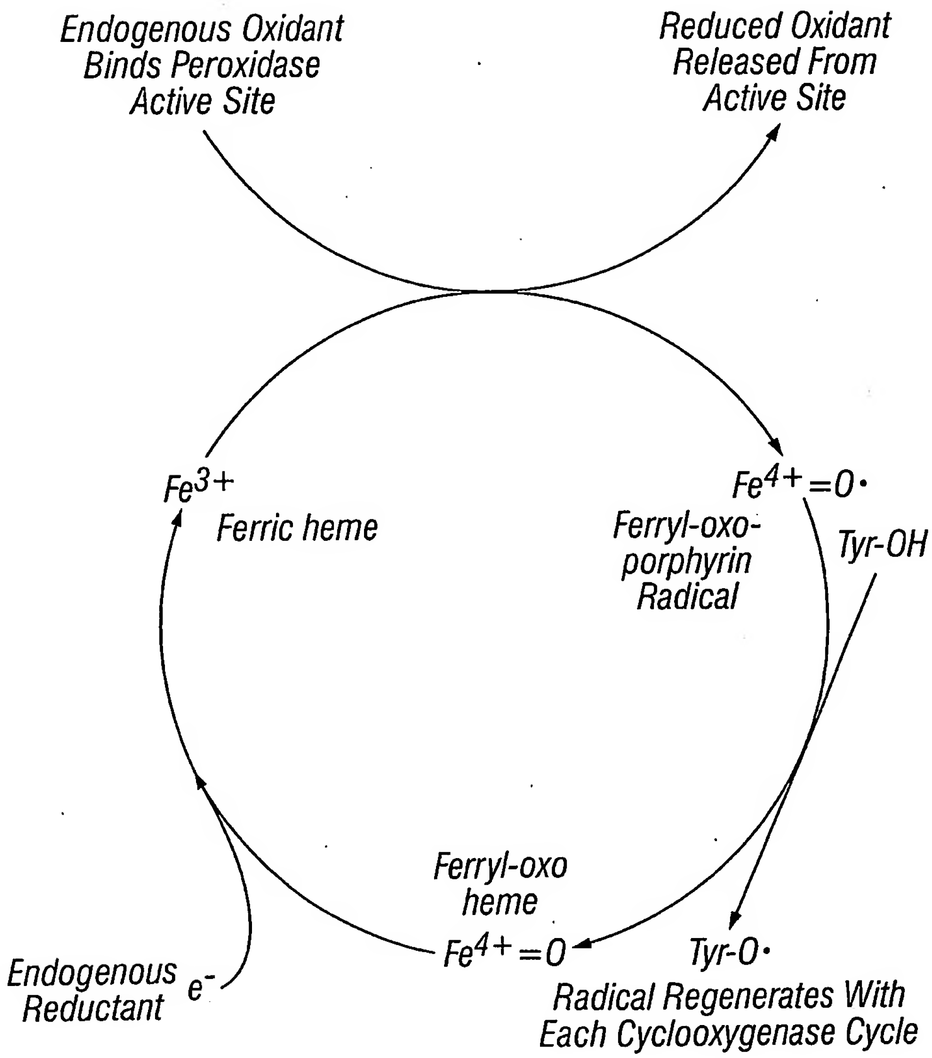
Endogenous Oxidant Binds Peroxidase Active Site
Reduced Oxidant Released From Active Site
Fe^{3+}
Ferric heme
$Fe^{4+}=O\cdot$
Ferryl-oxo-porphyrin Radical
Tyr-OH
Ferryl-oxo heme
$Fe^{4+}=O$
Tyr-O·
Radical Regenerates With Each Cyclooxygenase Cycle
Endogenous Reductant
e^-

FIG. 4

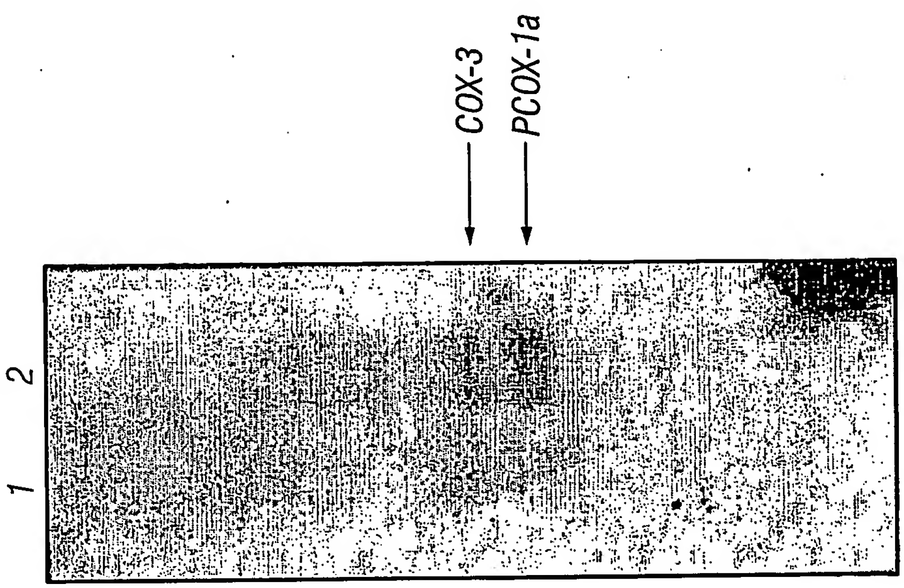

FIG. 5C

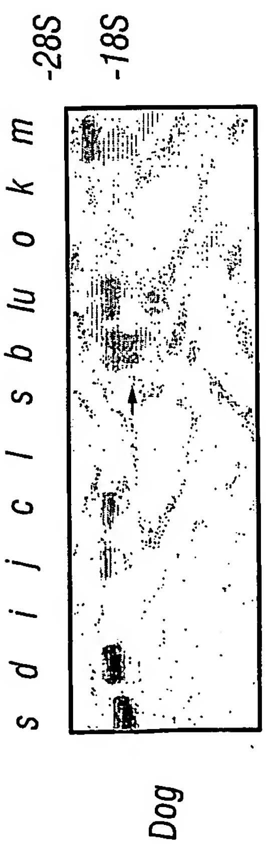

FIG. 5A

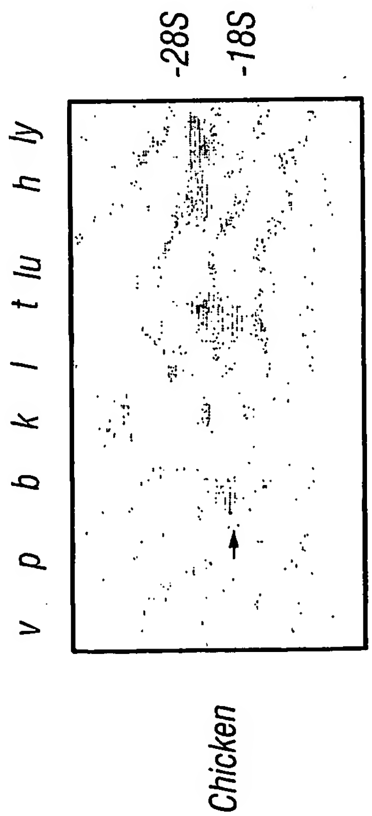

FIG. 5B

```
           1        10        20        30        40        50        60        70        80        90       100       110       120       130
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine                                                               GCCGCCCAGAGCTATGAGCCG-----------------------------------------------------
PCOX-1                                                CGGCGAGCGCAGCAGCCGCCCAGAGCTATGAGCCGTGAGTTCGACCCTGAGGCCCCCAGGAACCCTCTTCGCCTCCCGGGGGAGCC
PCOX-1d657 CGGGCAGCTCCTGGCACCGGCGCCCCGGGAGCCCGCAGTCTGCACCCCGAGCGCAGCAGCCGCCCAGAGCTATGAGCCGTGAGTTCGACCCTGAGGCCCCCAGGAACCCTCTTCGCCTCCCGGGGGAGCC
Consensus  .............................................c..cgagcgcagcaGCCGCCCAGAGCTATGAGCCGtgagttcgaccctgaggcccccaggaaccctcttcgcctcccgggggagcc

         131       140       150       160       170       180       190       200       210       220       230       240       250       260
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     --------------------------------------GGGGAGTCGCCTGCACCGGTGGCCGCTGCTCCTGCTGCTGCTGCTGCTGCTCCCGCCGCCCCCGGTCCTGCCCGCGGAAGCCCGGACCCCG
PCOX-1     TCGAATGCCAGGCCCAGCCCTCACCTCTCGCTCCGCAGGGGGGAGTCGCCTGCACCGGTGGCCGCTGCTCCTGCTGCTGCTGCTTGCTGCTCCGCCGCCCCCGGTCCTGCCCGCGGAAGCCCGGACCCCG
PCOX-1d657 TCGAATGCCAGGCCCAGCCCTCACCTCTCGCTCCGCAGGGGGGAGTCGCCTGCACCGGTGGCCGCTGCTCCTGCTGCTGCTGCTTGCTGCTCCGCCGCCCCCGGTCCTGCCCGCGGAAGCCCGGACCCCG
Consensus  tcgaatgccaggcccagccctcacctctcgctccgcaggGGGGAGTCGCCTGCACCGGTGGCCGCTGCTCCTGCTGCTGCTGCTTGCTGCTCCGCCGCCCCCGGTCCTGCCCGCGGAAGCCCGGACCCCG

         261       270       280       290       300       310       320       330       340       350       360       370       380       390
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     GCGCCTGTGAACCCGTGTTGTTACTACCCATGTCAGCACCAAGGGATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGTGTGACTGCACCCGCACGGGCTATTCTGGCCCCAACTGCACCATCCCCGAGC
PCOX-1     GCGCCTGTGAACCCGTGTTGTTACTACCCATGTCAGCACCAAGGGATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGTGTGACTGCACCCGCACGGGCTATTCTGGCCCCAACTGCACCATCCCCGAGC
PCOX-1d657 GCGCCTGTGAACCCGTGTTGTTACTACCCATGTCAGCACCAAGGGATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGTGTGACTGCACCCGCACGGGCTATTCTGGCCCCAACTGCACCATCCCCGAGC
Consensus  GCGCCTGTGAACCCGTGTTGTTACTACCCATGTCAGCACCAAGGGATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGTGTGACTGCACCCGCACGGGCTATTCTGGCCCCAACTGCACCATCCCCGAGC

         391       400       410       420       430       440       450       460       470       480       490       500       510       520
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     TGTGGACCTGGCTCCGGAATTCACTGCGCCCCAGTCCCTCTTTCCTCCACTTCCTGCTGACGCATGGGCGCTGGTTTTGGGAATTCATCAATGCCACCTTCATCCGTGACATGCTCATGCGTCTGGTACT
PCOX-1     TGTGGACCTGGCTCCGGAATTCACTGCGCCCCAGTCCCTCTTTCCTCCACTTCCTGCTGACGCATGGGCGCTGGTTTTGGGAATTCATCAATGCCACCTTCATCCGTGACATGCTCATGCGTCTGGTACT
PCOX-1d657 TGTGGACCTGGCTCCGGAATTCACTGCGCCCCAGTCCCTCTTTCCTCCACTTCCTGCTGACGCATGGGCGCTGGTTTTGGGAATTCATCAATGCCACCTTCATCCGTGACATGCTCATGCGTCTGGTACT
Consensus  TGTGGACCTGGCTCCGGAATTCACTGCGCCCCAGTCCCTCTTTCCTCCACTTCCTGCTGACGCATGGGCGCTGGTTTTGGGAATTCATCAATGCCACCTTCATCCGTGACATGCTCATGCGTCTGGTACT
```

FIG. 6A-1

```
            521      530       540       550       560       570       580       590       600       610       620       630       640       650
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine      CACAGCGCGTTCCAACCTTATCCCCAGTCCTCCCACCTACAACATAGCGCATGACTACATCAGCTGGGAGTCCTTCTCCAATGTGAGCTATTACACTCGTGTTCTGCCCTCTGTGCCCCAAGATTGCCCC
PCOX-1      CACAGCGCGTTCCAACCTTATCCCCAGTCCTCCCACCTACAACATAGCGCATGACTACATCAGCTGGGAGTCCTTCTCCAATGTGAGCTATTACACTCGTGTTCTGCCCTCTGTGCCCCAAGATTGCCCC
PCOX-1d657  CACAG-----------------------------------------------------------------------------------------------------------------------------
Consensus   CACAGcgcgttccaaccttatccccagtcctcccacctacaacatagcgcatgactacatcagctgggagtccttatccaatgtgagctattacactcgtgttctgaaatctgtgccccaagattgcccc
            651      660       670       680       690       700       710       720       730       740       750       760       770       780
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine      ACGCCCATGGGGACCAAAGGGAAGAAGCAGTTGCCAGACGCCCAACTCCTGGGCCGTCGCTTCCTGCTCAGGAGGAAGTTCATACCTGACCCCCAAGGCACCAACCTCATGTTCGCCTTCTTTGCACAAC
PCOX-1      ACGCCCATGGGGACCAAAGGGAAGAAGCAGTTGCCAGACGCCCAACTCCTGGGCCGTCGCTTCCTGCTCAGGAGGAAGTTCATACCTGACCCCCAAGGCACCAACCTCATGTTCGCCTTCTTTGCACAAC
PCOX-1d657  ----------------------------------------------------------------------------------------------------------------------------------
Consensus   acgcccatggggaccaaagggaagaagcagttgccagacgcccaactcctgggccgtcgcttcctgctcaggaggaagttcatacctgacccccaaggcaccaacctcatgttcgccttctttgcacaac
            781      790       800       810       820       830       840       850       860       870       880       890       900       910
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine      ACTTCACCCATCAGTTCTTCAAAACTTCTGGCAAGATGGGTCCTGGCTTCACCAAGGCCTTGGGCCATGGGGTAGATCTTGGCCACATTTATGGGGACAATCTGGACCGTCAGTATCAGCTGCGGCTCTT
PCOX-1      ACTTCACCCATCAGTTCTTCAAAACTTCTGGCAAGATGGGTCCTGGCTTCACCAAGGCCTTGGGCCATGGGGTAGATCTTGGCCACATTTATGGGGACAATCTGGACCGTCAGTATCAGCTGCGGCTCTT
PCOX-1d657  ----------------------------------------------------------------------------------------------------------------------------------
Consensus   acttcacccatcagttattcaaaacttctggcaagatgggtcctggcttcaccaaggccttgggccatggggtagatcttggccacatttatggggacaatatggaccgtcagtatcagctgcggctctt
            911      920       930       940       950       960       970       980       990       1000      1010      1020      1030      1040
            |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine      TAAGGATGGGAAACTCAAGTATCAGGTTCTGGATGGAGAGATGTACCCGCATCTGTGGAGGAGGCGCCTGTGTTGATGCACTACCCACGGGGCATTCTGCCCCAGAGTCAGATGGCCGTGGGCCAAGAA
PCOX-1      TAAGGATGGGAAACTCAAGTATCAGGTTCTGGATGGAGAGATGTACCCGCATCTGTGGAGGAGGCGCCTGTGTTGATGCACTACCCACGGGGCATTCTGCCCCAGAGTCAGATGGCCGTGGGCCAGGAG
PCOX-1d657  ----------------------------------------------------------------------------------------------------------------------------------
Consensus   taaggatgggaaactcaagtatcaggttatggatggagagatgtacccgccatctgtggaggaggagaatgtgttgatgcactacccacggggcattctgccccagagtcagatggccgtgggcca.ga.
```

FIG. 6A-2

```
           1041     1050      1060      1070      1080      1090      1100      1110      1120      1130      1140      1150      1160      1170
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     GTGTTTGGGCTGCTTCCTGGGCTCATGCTCTATGCCACGCTCTGGCTGCGTGAGCACAATCGTGTGTGTGACCTGCTGAAGGCTGAGCACCCCACTTGGGGTCATGAGCAACTGTTCCAGACGGCCCGAC
PCOX-1     GTGTTTGGGCTGCTTCCTGGGCTCATGCTCTATGCCACGCTCTGGCTGCGTGAGCACAATCGTGTGTGTGACCTGCTGAAGGCTGAGCACCCCACTTGGGGTCATGAGCAACTGTTCCAGACGGCCCGAC
PCOX-1d657 ----------------------------------------------------------------------------------------------------------------------------------
Consensus  gtgtttgggctgcttcctgggctcatgctctatgccacgctctggctgcgtgagcacaatcgtgtgtgtgacctgctgaaggctgagcaccccacttggggtcatgagcaactgttccagacggcccgac

           1171     1180      1190      1200      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     TCATCCTCATTGGGGAGACCATCAAGATTGTGATTGAGGAGTATGTGCAGCAGCTGAGTGGCTACTTCTTGCAGCTGAAGTTCGACCCGGAGCTGCTGTTTAGCGCCCAGTTCCAGTACCGCAACCGCAT
PCOX-1     TCATCCTCATTGGGGAGACCATCAAGATTGTGATTGAGGAGTATGTGCAGCAGCTGAGTGGCTACTTCTTGCAGCTGAAGTTCGACCCGGAGCTGCTGTTTAGCGCCCAGTTCCAGTACCGCAACCGCAT
PCOX-1d657 ------------GGGAGACCATCAAGATTGTGATTGAGGAGTATGTGCAGCAGCTGAGTGGCTACTTCTTGCAGCTGAAGTTCGACCCGGAGCTGCTGTTTAGCGCCCAGTTCCAGTACCGCAACCGCAT
Consensus  tcatcctcattgGGGAGACCATCAAGATTGTGATTGAGGAGTATGTGCAGCAGCTGAGTGGCTACTTCTTGCAGCTGAAGTTCGACCCGGAGCTGCTGTTTAGCGCCCAGTTCCAGTACCGCAACCGCAT

           1301     1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     CGCCATGGAGTTCAACCAGCTGTACCACTGGCACCCGCTCATGCCAGACTCCTTCTGGGTGGGTTCCCAGGAGTACAGCTATCAGCAGTTCCTGTTCAACACCTCCATGCTGACGCACTACGGGATCGAG
PCOX-1     CGCCATGGAGTTCAACCAGCTGTACCACTGGCACCCGCTCATGCCAGACTCCTTCTGGGTGGGTTCCCAGGAGTACAGCTATCAGCAGTTCCTGTTCAACACCTCCATGCTGACGCACTACGGGATCGAG
PCOX-1d657 CGCCATGGAGTTCAACCAGCTGTACCACTGGCACCCGCTCATGCCAGACTCCTTCTGGGTGGGTTCCCAGGAGTACAGCTATCAGCAGTTCCTGTTCAACACCTCCATGCTGACGCACTACGGGATCGAG
Consensus  CGCCATGGAGTTCAACCAGCTGTACCACTGGCACCCGCTCATGCCAGACTCCTTCTGGGTGGGTTCCCAGGAGTACAGCTATCAGCAGTTCCTGTTCAACACCTCCATGCTGACGCACTACGGGATCGAG

           1431     1440      1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     GCCCTGGTGGATGCCTTCTCTCGCCAGAGCGCCGGCCGGATTGGTGGAGGTAGAAACATAGACCACCATGTCCTGCACGTGGCTGTGGAAACCATCAAGGAATCCCGCGAGTTGCGGCTGCAGCCCTTCA
PCOX-1     GCCCTGGTGGATGCCTTCTCTCGCCAGAGCGCCGGCCGGATTGGTGGAGGTAGAAACATAGACCACCATGTCCTGCACGTGGCTGTGGAAACCATCAAGGAATCCCGCGAGTTGCGGCTGCAGCCCTTCA
PCOX-1d657 GCCCTGGTGGATGCCTTCTCTCGCCAGAGCGCCGGCCGGATTGGTGGAGGTAGAAACATAGACCACCATGTCCTGCACGTGGCTGTGGAAACCATCAAGGAATCCCGCGAGTTGCGGCTGCAGCCCTTCA
Consensus  GCCCTGGTGGATGCCTTCTCTCGCCAGAGCGCCGGCCGGATTGGTGGAGGTAGAAACATAGACCACCATGTCCTGCACGTGGCTGTGGAAACCATCAAGGAATCCCGCGAGTTGCGGCTGCAGCCCTTCA

           1561     1570      1580      1590      1600      1610      1620      1630      1640      1650      1660      1670      1680      1690
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     ATGAGTACCGCAAGAGGTTTGGCATGAGGCCCTACATGTCCTTCCAGGAACTCACAGGGGAGAAGGAGATGGCAGCCGAGTTGGAGGAGCTGTATGGAGACATTGATGCCTTGGAATTCTACCCGGGGCT
PCOX-1     ATGAGTACCGCAAGAGGTTTGGCATGAGGCCCTACATGTCCTTCCAGGAACTCACAGGGGAGAAGGAGATGGCAGCCGAGTTGGAGGAGCTGTATGGAGACATTGATGCCTTGGAATTCTACCCGGGGCT
PCOX-1d657 ATGAGTACCGCAAGAGGTTTGGCATGAGGCCCTACATGTCCTTCCAGGAACTCACAGGGGAGAAGGAGATGGCAGCCGAGTTGGAGGAGCTGTATGGAGACATTGATGCCTTGGAATTCTACCCGGGGCT
Consensus  ATGAGTACCGCAAGAGGTTTGGCATGAGGCCCTACATGTCCTTCCAGGAACTCACAGGGGAGAAGGAGATGGCAGCCGAGTTGGAGGAGCTGTATGGAGACATTGATGCCTTGGAATTCTACCCGGGGCT
```

FIG. 6A-3

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           1691      1700      1710      1720      1730      1750      1740      1760      1770      1780      1790      1800      1810      1820
           |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     TCTTCTGGAGAAGTGCCATCCAAACTCCATCTTTGGAGAGAGTATGATAGAAATTGGGGCTCCCTTCTCCCTTAAGGGCCTCCTAGGGAATCCCATCTGTTCTCCAGAGTACTGGAAGCCAAGCACATTC
PCOX-1     TCTTCTGGAGAAGTGCCATCCAAACTCCATCTTTGGAGAGAGTATGATAGAAATTGGGGCTCCCTTCTCCCTTAAGGGCCTCCTAGGGAATCCCATCTGTTCTCCAGAGTACTGGAAGCCAAGCACATTC
PCOX-1d657 TCTTCTGGAGAAGTGCCATCCAAACTCCATCTTTGGAGAGAGTATGATAGAAATTGGGGCTCCCTTCTCCCTTAAGGGCCTCCTAGGGAATCCCATCTGTTCTCCAGAGTACTGGAAGCCAAGCACATTC
Consensus  TCTTCTGGAGAAGTGCCATCCAAACTCCATCTTTGGAGAGAGTATGATAGAAATTGGGGCTCCCTTCTCCCTTAAGGGCCTCCTAGGGAATCCCATCTGTTCTCCAGAGTACTGGAAGCCAAGCACATTC
           1821      1830      1840      1850      1860      1880      1870      1890      1900      1910      1920      1930      1940      1950
           |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     GGTGGTGAGATGGGCTTCAATATGGTCAAGACAGCCACACTGAAGAAGCTGGTCTGCCTTAACACCAAGACTTGTCCCTATGTTTCCTTCCGTGTGCCTGACCCCCACCAGGATGGCGGGCCTGGTGTGG
PCOX-1     GGTGGTGAGATGGGCTTCAATATGGTCAAGACAGCCACACTGAAGAAGCTGGTCTGCCTTAACACCAAGACTTGTCCCTATGTTTCCTTCCGTGTGCCTGACCCCCACCAGGATGGCGGGCCTGGTGTGG
PCOX-1d657 GGTGGTGAGATGGGCTTCAATATGGTCAAGACAGCCACACTGAAGAAGCTGGTCTGCCTTAACACCAAGACTTGTCCCTATGTTTCCTTCCGTGTGCCTGACCCCCACCAGGATGGCGGGCCTGGTGTGG
Consensus  GGTGGTGAGATGGGCTTCAATATGGTCAAGACAGCCACACTGAAGAAGCTGGTCTGCCTTAACACCAAGACTTGTCCCTATGTTTCCTTCCGTGTGCCTGACCCCCACCAGGATGGCGGGCCTGGTGTGG
           1951      1960      1970      1980      1990      2010      2000      2020      2030      2040      2050      2060      2070      2080
           |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     AGCGGCCGTCCACAGAGCTCTGAGGGGGCAGAGCAGCAGCATTCTGGAGGGTGGACTTGTCATCCCAGAATGCTGAGGCTGGGGTTAATAATCCCAAATGTTGGGTCTTTGGTTTGCCTCAAGAATATCA
PCOX-1     AGCGGCCGTCCACAGAGCTCTGAGGGGGCAGAGCAGCAGCATTCTGGAGGGTGGACTTGTCATCCCAGAATGCTGAGGCTGGGGTTAATAATCCCAAATGTTGGGTCTTTGGTTTGCCTCAAGAATATCA
PCOX-1d657 AGCGGCCGTCCACAGAGCTCTGAGGGGGCAGAGCAGCAGCATTCTGGAGGGTGGACTTGTCATCCCAGAATGCTGAGGCTGGGGTTAATAATCCCAAATGTTGGGTCTTTGGTTTGCCTCAAGAATATCA
Consensus  AGCGGCCGTCCACAGAGCTCTGAGGGGGCAGAGCAGCAGCATTCTGGAGGGTGGACTTGTCATCCCAGAATGCTGAGGCTGGGGTTAATAATCCCAAATGTTGGGTCTTTGGTTTGCCTCAAGAATATCA

           2001      2030      2100      2110      2120      2140      2130      2150      2160      2170      2180      2190      2200      2210
           |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     AGGTCAACATTTAGAACTTTGTGTCTCTCACCCATTATCTGGAATATCATGGTCTTGTTTGTTATTCTAGAATGCTGAATTCCTGGTTGACCATCTAGAATGGATGGAGTGATGCTTCTTTGGCAAGCCA
PCOX-1     AGGTCAACATTTAGAACTTTGTGTCTCTCACCCATTATCTGGAATATCATGGTCTTGTTTGTTATTCTAGAATGCTGAATTCCTGGTTGACCATCTAGAATGGATGGAGTGATGCTTCTTTGGCAAGCCA
PCOX-1d657 AGGTCAACATTTAGAACTTTGTGTCTCTCACCCATTATCTGGAATATCATGGTCTTGTTTGTTATTCTAGAATGCTGAATTCCTGGTTGACCATCTAGAATGGATGGAGTGATGCTTCTTTGGCAAGCCA
Consensus  AGGTCAACATTTAGAACTTTGTGTCTCTCACCCATTATCTGGAATATCATGGTCTTGTTTGTTATTCTAGAATGCTGAATTCCTGGTTGACCATCTAGAATGGATGGAGTGATGCTTCTTTGGCAAGCCA
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FIG. 6A-4

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           2211     2220      2230      2240      2250      2260      2270      2280      2290      2300      2310      2320      2330      2340
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     GAACACTGGTTCCTGGCCGACAACCTAGAATGTCAGACTTCTGGTTGACTTAAGACGTAGGCATTCTCTAATGTGAAGCTCCTGACAGAATCATCTAGAAAGATAGGGGATTCTTATTTTGCATTCTAGA
PCOX-1     GAACACTGGTTCCTGGCCGACAACCTAGAATGTCAGACTTCTGGTTGACTTAAGACGTAGGCATTCTCTAATGTGAAGCTCCTGACAGAATCATCTAGAAAGATAGGGGATTCTTATTTTGCATTCTAGA
PCOX-1d657 GAACACTGGTTCCTGGCCGACAACCTAGAATGTCAGACTTCTGGTTGACTTAAGACGTAGGCATTCTCTAATGTGAAGCTCCTGACAGAATCATCTAGAAAGATAGGGGATTCTTATTTTGCATTCTAGA
Consensus  GAACACTGGTTCCTGGCCGACAACCTAGAATGTCAGACTTCTGGTTGACTTAAGACGTAGGCATTCTCTAATGTGAAGCTCCTGACAGAATCATCTAGAAAGATAGGGGATTCTTATTTTGCATTCTAGA
           2341     2350      2360      2370      2380      2390      2400      2410      2420      2430      2440      2450      2460      2470
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     ATTCTGGGCAGCCCTCCAGCATGTTGATTTTTTTCACTGGCAGTTCAGAATGTTGTGCTCTTGATTGCTGATCCAAAATAGTGGCTGGTATGCCAGATCAGTCTTGCTCTGAATGCCTAGAATGGTAATT
PCOX-1     ATTCTGGGCAGCCCTCCAGCATGTTGATTTTTTTCACTGGCAGTTCAGAATGTTGTGCTCTTGATTGCTGATCCAAAATAGTGGCTGGTATGCCAGATCAGTCTTGCTCTGAATGCCTAGAATGGTAATT
PCOX-1d657 ATTCTGGGCAGCCCTCCAGCATGTTGATTTTTTTCACTGGCAGTTCAGAATGTTGTGCTCTTGATTGCTGATCCAAAATAGTGGCTGGTATGCCAGATCAGTCTTGCTCTGAATGCCTAGAATGGTAATT
Consensus  ATTCTGGGCAGCCCTCCAGCATGTTGATTTTTTTCACTGGCAGTTCAGAATGTTGTGCTCTTGATTGCTGATCCAAAATAGTGGCTGGTATGCCAGATCAGTCTTGCTCTGAATGCCTAGAATGGTAATT

           2471     2480      2490      2500      2510      2520      2530      2540      2550      2560      2570      2580      2590      2600
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     TGATTCATTTTCCTGTTCAGTGAGATACCCCCAAAGCAGGAGAATCTACAGCCTAACCACAGTGCATTGCCTGCCTCTGTGCCTGCCCCAAGGACTTAGGGGGCAGAGTGTTCTTCCTGGGATGCTGACT
PCOX-1     TGATTCATTTTCCTGTTCAGTGAGATACCCCCAAAGCAGGAGAATCTACAGCCTAACCACAGTGCATTGCCTGCCTCTGTGCCTGCCCCAAGGACTTAGGGGGCAGAGTGTTCTTCCTGGGATGCTGACT
PCOX-1d657 TGATTCATTTTCCTGTTCAGTGAGATACCCCCAAAGCAGGAGAATCTACAGCCTAACCACAGTGCATTGCCTGCCTCTGTGCCTGCCC
Consensus  TGATTCATTTTCCTGTTCAGTGAGATACCCCCAAAGCAGGAGAATCTACAGCCTAACCACAGTGCATTGCCTGCCTCTGTGCCTGCCCc.aggacttagggggcagagtgttcttcctggga.gctgact

           2601     2610      2620      2630      2640      2650      2660      2670      2680      2690      2700      2710      2720      2730
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Canine     CAGACCCTGGTCCAAGGAGATAGAACAGGTGGGCTTTTTCCAGGTCATTGGTTGGAGGCCACCAGAGCTCTGTTGCCATCTTTGTCTTGACTCATGACAGCTGTTTCTCATGAAACTAATAAAATTTTTT
PCOX-1     CAGACCCTGGTCCAAGGAGCTAGAACAGGTGGGCTTTTTCCAGGTCATTGGTTGGAGGCCACCAGAGCTCTGTTGCCATCTTTGTCTTGACTCATGACAGCTGTTTCTCATGAAACTAATAAAATTCTTT
PCOX-1d657
Consensus  cagaccctggtccaaggag.tagaacaggtgggctttttccaggtcattggttggaggccaccagagctctgttgccatctttgtcttgactcatgacagctgtttctcatgaaactaataaaatt.ttt

           2731     2740      2750
           |--------+---------|
Canine     TTCCCTCTTTTC
PCOX-1     TTCCAAAAAAAAAAAAAAAA
PCOX-1d657
Consensus  ttcc................
```

FIG. 6A-5

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```
                1        10        20        30        40        50        60        70        80        90        100       110       120       130
Canine          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
(Genebank)                                    MSRGSRLHRWPLLLLLLLLLPPPPVLPAEARTPAPVNPCCYYPCQHQGICVRFGLDRYQCDCTRTGYSGPNCTIPELWTWLRHSLRPSPSFLHFLLTHGR
PCOX-1          MSREFDPEAPRNPLRLPGEPRMPGPALTSRSAGGSRLHRWPLLLLLLLLLPPPPVLPAEARTPAPVNPCCYYPCQHQGICVRFGLDRYQCDCTRTGYSGPNCTIPELWTWLRHSLRPSPSFLHFLLTHGR
PCOX-1d657      MSREFDPEAPRNPLRLPGEPRMPGPALTSRSAGGSRLHRWPLLLLLLLLLPPPPVLPAEARTPAPVNPCCYYPCQHQGICVRFGLDRYQCDCTRTGYSGPNCTIPELWTWLRHSLRPSPSFLHFLLTHGR
Consensus       msrefdpeaprnplrlpgeprmpgpaltsrsagGSRLHRWPLLLLLLLLLPPPPVLPAEARTPAPVNPCCYYPCQHQGICVRFGLDRYQCDCTRTGYSGPNCTIPELWTWLRHSLRPSPSFLHFLLTHGR
                131      140       150       160       170       180       190       200       210       220       230       240       250       260
Canine          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
(Genebank)      WFWEFINATFIRDMLMRLVLTARSNLIPSPPTYNIAHDYISWESFSNVSYYTRVLPSVPQDCPTPMGTKGKKQLPDAQLLGRRFLLRRKFIPDPQGTNLMFAFFAQHFTHQFFKTSGKMGPGFTKALGHG
PCOX-1          WFWEFINATFIRDMLMRLVLTARSNLIPSPPTYNIAHDYISWESFSNVSYYTRVLPSVPQDCPTPMGTKGKKQLPDAQLLGRRFLLRRKFIPDPQGTNLMFAFFAQHFTHQFFKTSGKMGPGFTKALGHG
PCOX-1d657      WFWEFINATFIRDMLMRLVLT--------------------------------------------------------------------------------------------------------------
Consensus       WFWEFINATFIRDMLMRLVLTarsnlipspptyniahdyiswesfsnvsyytrvlpsvpqdcptpmgtkgkkqlpdaqllgrrfllrrkfipdpqgtnlmfaffaqhfthqffktsgkmgpgftkalghg
                261      270       280       290       300       310       320       330       340       350       360       370       380       390
Canine          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
(Genebank)      VDLGHIYGDNLDRQYQLRLFKDGKLKYQVLDGEMYPPSVEEAPVLMHYPRGILPQSQMAVGQEVFGLLPGLMLYATLWLREHNRVCDLLKAEHPTWGDEQLFQTARLILIGETIKIVIEEYVQQLSGYFL
PCOX-1          VDLGHIYGDNLDRQYQLRLFKDGKLKYQVLDGEMYPPSVEEAPVLMHYPRGILPQSQMAVGQEVFGLLPGLMLYATLWLREHNRVCDLLKAEHPTWGDEQLFQTARLILIGETIKIVIEEYVQQLSGYFL
PCOX-1d657      ----------------------------------------------------------------------------------------------------------GETIKIVIEEYVQQLSGYFL
Consensus       vdlghiygdnldrqyqlrlfkdgklkyqvldgemyppsveeapvlmhyprgilpqsqmavgqevfgllpglmlyatlwlrehnrvcdllkaehptwgdeqlfqtarLILIGETIKIVIEEYVQQLSGYFL
                391      400       410       420       430       440       450       460       470       480       490       500       510       520
Canine          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
(Genebank)      QLKFDPELLFSAQFQYRNRIAMEFNQLYHWHPLMPDSFWVGSQEYSYEQFLFNISMLTHYGIEALVDAFSRQSAGRIGGGRNIDHHVLHVAVETIKESRELRLQPFNEYRKRFGMRPYMSFQELTGEKEM
PCOX-1          QLKFDPELLFSAQFQYRNRIAMEFNQLYHWHPLMPDSFWVGSQEYSYEQFLFNISMLTHYGIEALVDAFSRQSAGRIGGGRNIDHHVLHVAVETIKESRELRLQPFNEYRKRFGMRPYMSFQELTGEKEM
PCOX-1d657      QLKFDPELLFSAQFQYRNRIAMEFNQLYHWHPLMPDSFWVGSQEYSYEQFLFNISMLTHYGIEALVDAFSRQSAGRIGGGRNIDHHVLHVAVETIKESRELRLQPFNEYRKRFGMRPYMSFQELTGEKEM
Consensus       QLKFDPELLFSAQFQYRNRIAMEFNQLYHWHPLMPDSFWVGSQEYSYEQFLFNISMLTHYGIEALVDAFSRQSAGRIGGGRNIDHHVLHVAVETIKESRELRLQPFNEYRKRFGMRPYMSFQELTGEKEM
                521      530       540       550       560       570       580       590       600       610       620       630 634
Canine          |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---|
(Genebank)      AAELEELYGDIDALEFYPGLLLEKCHPNSIFGESMIEIGAPFSLKGLLGNPICSPEYWKPSTFGGEMGFNMVKTATLKKLVCLNTKTCPYVSFRVPDPHQDGGPGVERPSTELZ
PCOX-1          AAELEELYGDIDALEFYPGLLLEKCHPNSIFGESMIEIGAPFSLKGLLGNPICSPEYWKPSTFGGEMGFNMVKTATLKKLVCLNTKTCPYVSFRVPDPHQDGGPGVERPSTELZ
PCOX-1d657      AAELEELYGDIDALEFYPGLLLEKCHPNSIFGESMIEIGAPFSLKGLLGNPICSPEYWKPSTFGGEMGFNMVKTATLKKLVCLNTKTCPYVSFRVPDPHQDGGPGVERPSTELZ
Consensus       AAELEELYGDIDALEFYPGLLLEKCHPNSIFGESMIEIGAPFSLKGLLGNPICSPEYWKPSTFGGEMGFNMVKTATLKKLVCLNTKTCPYVSFRVPDPHQDGGPGVERPSTELZ
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FIG. 6B

```
           1        10        20        30        40        50        60        70        80        90        101
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------||
Canine     GTGAGTTCGACCCTGAGGC--------CCCCAGG--AACCCTCTTCGCCTCCCGGGGGAGCCTCGAATGCCAGGCCCAGCCCTCACCTCTCGCTCCGCAG
Human      GTGAGTGCGACCCCGGTGC--------CCGGTGGGGAATTTTCTTGGCCTCCTGGTGGAGCCTTGAATGCCAGCTCAGCCCCTCATCTCTCTCCTCTGCAG
Murine     GTGAGTCCGACCCCAGTGGTGCCCCCACGCGTCCCGGAATTCGGTGGCCTGCAGGCGGAGCCTTGAACGCTAGGCTCAACTCTCTCTTCCTTCTGCAGGAA
Consensus  GTGAGT.CGACCCcggtGc........Cccgtgg.gaA.tttctTgGCCTcC.GG.GGAGCCTtGAAtGCcAGgccca.CcCTCaccTCtctCt.C.g.a.

           1        10        20        30  34
           |--------+---------+---------+---|
Canine     REFDPEAP--RNPLRLPGEPRMPGPALTSRSA
Human      RECDPGA---RWGIFLASWWSLECQLSPSSLSSA
Murine     RESDPSGAPTRPGIRWPAGGALNARLNSLFLLQE
Consensus  RE.DP:a...R.girlp....$...l..s.l...
```

FIG. 7

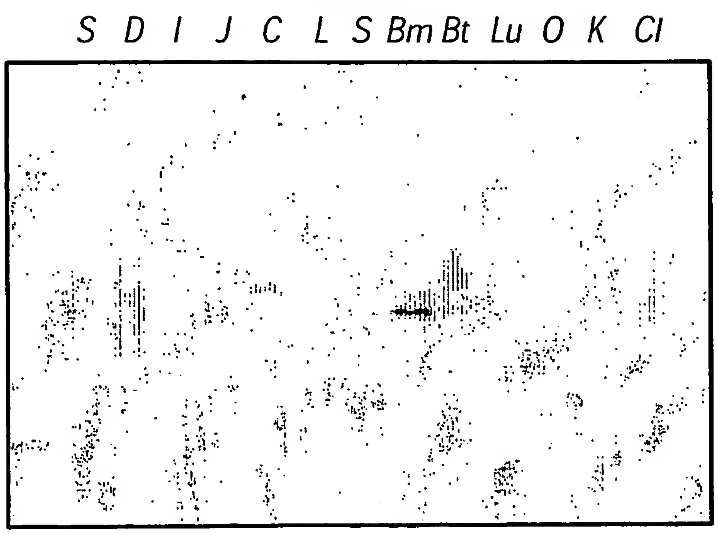

FIG. 8

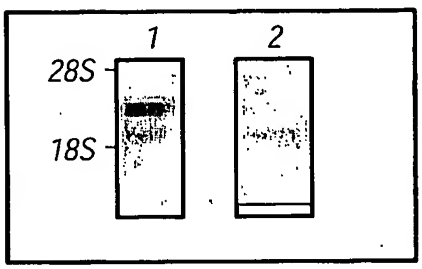

FIG. 10A

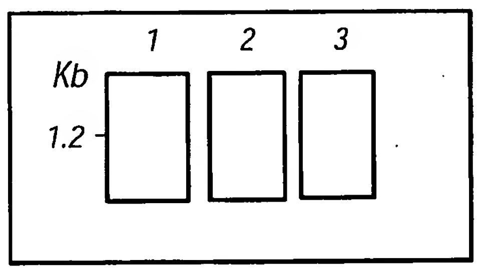

FIG. 10B

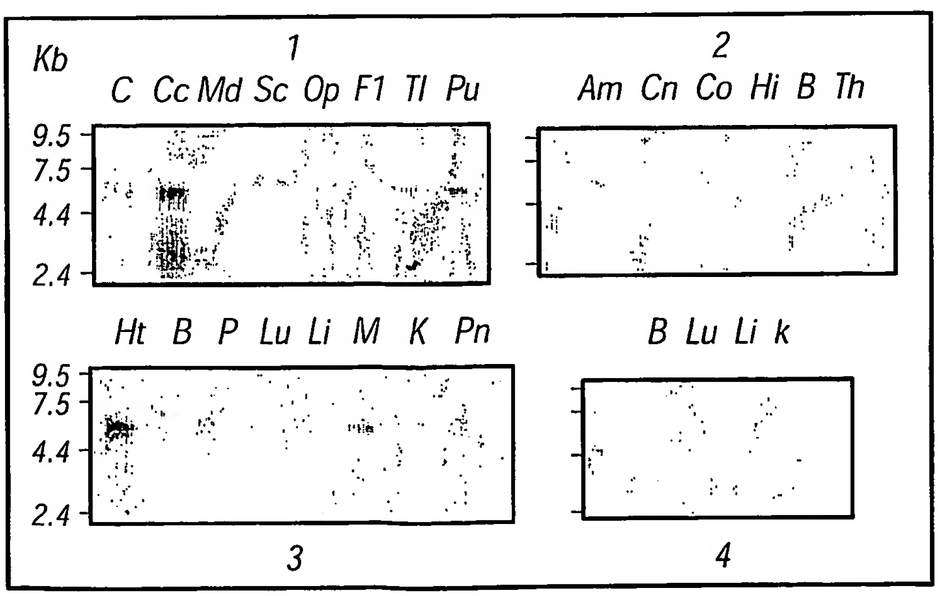

FIG. 10C

SEQ ID NO:1 1 ATGAGCCGTG AGTTCGACCC TGAGGCCCCC AGGAACCCTC TTCGCCTCCC GGGGGAGCCT 60
61 CGAATGCCAG GCCCAGCCCT CACCTCTCGC TCCGCAGGGG GGAGTCGCCT GCACCGGTGG 120
121 CCGCTGCTCC TGCTGCTGCT GCTGCTGCTC CCGCCGCCCC CGGTCCTGCC CGCGGAAGCC 180
181 CGGACCCCGG CGCCTGTGAA CCCGTGTTGT TACTACCCAT GTCAGCACCA AGGGATCTGT 240
241 GTCCGCTTCG GCCTTGACCG CTACCAGTGT GACTGCACCC GCACGGGCTA TTCTGGCCCC 300
301 AACTGCACCA TCCCCGAGCT GTGGACCTGG CTCCGGAATT CACTGCGCCC CAGTCCCTCT 360
361 TTCCTCCACT TCCTGCTGAC GCATGGGCGC TGGTTTTGGG AATTCATCAA TGCCACCTTC 420
421 ATCCGTGACA TGCTCATGCG TCTGGTACTC ACAGCGCGTT CCAACCTTAT CCCCAGTCCT 480
481 CCCACCTACA ACATAGCGCA TGACTACATC AGCTGGGAGT CCTTCTCCAA TGTGAGCTAT 540
541 TACACTCGTG TTCTGCCCTC TGTGCCCCAA GATTGCCCCA CGCCCATGGG GACCAAAGGG 600
601 AAGAAGCAGT TGCCAGACGC CCAACTCCTG GGCCGTCGCT TCCTGCTCAG GAGGAAGTTC 660
661 ATACCTGACC CCCAAGGCAC CAACCTCATG TTCGCCTTCT TTGCACAACA CTTCACCCAT 720
721 CAGTTCTTCA AAACTTCTGG CAAGATGGGT CCTGGCTTCA CCAAGGCCTT GGGCCATGGG 780
781 GTAGATCTTG GCCACATTTA TGGGGACAAT CTGGACCGTC AGTATCAGCT GCGGCTCTTT 840
841 AAGGATGGGA AACTCAAGTA TCAGGTTCTG GATGGAGAGA TGTACCCGCC ATCTGTGGAG 900
901 GAGGCGCCTG TGTTGATGCA CTACCCACGG GGCATTCTGC CCCAGAGTCA GATGGCCGTG 960
961 GGCCAGGAGG TGTTTGGGCT GCTTCCTGGG CTCATGCTCT ATGCCACGCT CTGGCTGCGT 1020

FIG. 9A-1

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1021 GAGCACAATC GTGTGTGTGA CCTGCTGAAG GCTGAGCACC CCACTTGGGG TGATGAGCAA 1080
1081 CTCTTCCAGA CGGCCCGACT CATCCTCATT GGGGAGACCA TCAAGATTGT GATTGAGGAG 1140
1141 TATGTGCAGC AGCTGAGTGG CTACTTCTTG CAGCTGAAGT TCGACCCGGA GCTGCTGTTT 1200
1201 AGCGCCCAGT TCCAGTACCG CAACCGCATC GCCATGGAGT TCAACCAGCT GTACCACTGG 1260
1261 CACCCGCTCA TGCCAGACTC CTTCTGGGTG GGTTCCCAGG AGTACAGCTA TGAGCAGTTC 1320
1321 CTGTTCAACA CCTCCATGCT GACGCACTAC GGGATCGAGG CCCTGGTGGA TGCCTTCTCT 1380
1381 CGCCAGAGCG CCGGCCGGAT TGGTGGAGGT AGAAACATAG ACCACCATGT CCTGCACGTG 1440
1441 GCTGTGGAAA CCATCAAGGA ATCCCGCGAG TTGCGGCTGC AGCCCTTCAA TGAGTACCGC 1500
1501 AAGAGGTTTG GCATGAGGCC CTACATGTCC TTCCAGGAAC TCACAGGGGA GAAGGAGATG 1560
1561 GCAGCCGAGT TGGAGGAGCT GTATGGAGAC ATTGATGCCT TGGAATTCTA CCCGGGGCTT 1620
1621 CTTCTGGAGA AGTGCCATCC AAACTCCATC TTTGGAGAGA GTATGATAGA AATTGGGGCT 1680
1681 CCCTTCTCCC TTAAGGGCCT CCTAGGGAAT CCCATCTGTT CTCCAGAGTA CTGGAAGCCA 1740
1741 AGCACATTCG GTGGTGAGAT GGGCTTCAAT ATGGTCAAGA CAGCCACACT GAAGAAGCTG 1800
1801 GTCTGCCTTA ACACCAAGAC TTGTCCCTAT GTTTCCTTCC GTGTGCCTGA CCCCCACCAG 1860
1861 GATGGCGGGC CTGGTGTGCA GCGGCCGTCC ACAGAGCTCT GA                    1902
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FIG. 9A-2

```
SEQ ID NO:2   1 MSREFDPEAP RNPLRLPGEP RMPGPALTSR SAGGSRLHRW PLLLLLLLLL PPPPVLPAEA  60
             61 RTPAPVNPCC YYPCQHQGIC VRFGLDRYQC DCTRTGYSGP NCTIPELWTW LRNSLRPSPS 120
            121 FLHFLLTHGR WFWEFINATF IRDMLMRLVL TARSNLIPSP PTYNIAHDYI SWESFSNVSY 180
            181 YTRVLPSVPQ DCPTPMGTKG KKQLPDAQLL GRRFLLRRKF IPDPQGTNLM FAFFAQHFTH 240
            241 QFFKTSGKMG PGFTKALGHG VDLGHIYGDN LDRQYQLRLF KDGKLKYQVL DGEMYPPSVE 300
            301 EAPVLMHYPR GILPQSQMAV GQEVFGLLPG LMLYATLWLR EHNRVCDLLK AEHPTWGDEQ 360
            361 LFQTARLILI GETIKIVIEE YVQQLSGYFL QLKFDPELLF SAQFQYRNRI AMEFNQLYHW 420
            421 HPLMPDSFWV GSQEYSYEQF LFNTSMLTHY GIEALVDAFS RQSAGRIGGG RNIDHHVLHV 480
            481 AVETIKESRE LRLQPFNEYR KRFGMRPYMS FQELTGEKEM AAELEELYGD IDALEFYPGL 540
            541 LLEKCHPNSI FGESMIEIGA PFSLKGLLGN PICSPEYWKP STFGGEMGFN MVKTATLKKL 600
            601 VCLNTKTCPY VSFRVPDPHQ DGGPGVQRPS TELZ                             634
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FIG. 9B

```
SEQ ID NO:3 1 CGGCGAGCGC AGCAGCCGCC CAGAGCTATG AGCCGTGAGT TCGACCCTGA GGCCCCCAGG 60
61 AACCCTCTTC GCCTCCCGGG GGAGCCTCGA ATGCCAGGCC CAGCCCTCAC CTCTCGCTCC 120
121 GCAGGGGGGA GTCGCCTGCA CCGGTGGCCG CTGCTCCTGC TGCTGCTGCT GCTGCTCCCG 180
181 CCGCCCCCGG TCCTGCCCGC GGAAGCCCGG ACCCCGGCGC CTGTGAACCC GTGTTGTTAC 240
241 TACCCATGTC AGCACCAAGG GATCTGTGTC CGCTTCGGCC TTGACCGCTA CCAGTGTGAC 300
301 TGCACCCGCA CGGGCTATTC TGGCCCCAAC TGCACCATCC CCGAGCTGTG GACCTGGCTC 360
361 CGGAATTCAC TGCGCCCCAG TCCCTCTTTC CTCCACTTCC TGCTGACGCA TGGGCGCTGG 420
421 TTTTGGGAAT TCATCAATGC CACCTTCATC CGTGACATGC TCATGCGTCT GGTACTCACA 480
481 GCGCGTTCCA ACCTTATCCC CAGTCCTCCC ACCTACAACA TAGCGCATGA CTACATCAGC 540
541 TGGGAGTCCT TCTCCAATGT GAGCTATTAC ACTCGTGTTC TGCCCTCTGT GCCCCAAGAT 600
601 TGCCCCACGC CCATGGGGAC CAAAGGGAAG AAGCAGTTGC CAGACGCCCA ACTCCTGGGC 660
661 CGTCGCTTCC TGCTCAGGAG GAAGTTCATA CCTGACCCCC AAGGCACCAA CCTCATGTTC 720
721 GCCTTCTTTG CACAACACTT CACCCATCAG TTCTTCAAAA CTTCTGGCAA GATGGGTCCT 780
781 GGCTTCACCA AGGCCTTGGG CCATGGGGTA GATCTTGGCC ACATTTATGG GGACAATCTG 840
841 GACCGTCAGT ATCAGCTGCG GCTCTTTAAG GATGGGAAAC TCAAGTATCA GGTTCTGGAT 900
901 GGAGAGATGT ACCCGCCATC TGTGGAGGAG GCGCCTGTGT TGATGCACTA CCCACGGGGC 960
961 ATTCTGCCCC AGAGTCAGAT GGCCGTGGGC CAGGAGGTGT TTGGGCTGCT TCCTGGGCTC 1020
1021 ATGCTCTATG CCACGCTCTG GCTGCGTGAG CACAATCGTG TGTGTGACCT GCTGAAGGCT 1080
1081 GAGCACCCCA CTTGGGGTGA TGAGCAACTC TTCCAGACGG CCCGACTCAT CCTCATTGGG 1140
1141 GAGACCATCA AGATTGTGAT TGAGGAGTAT GTGCAGCAGC TGAGTGGCTA CTTCTTGCAG 1200
1201 CTGAAGTTCG ACCCGGAGCT GCTGTTTAGC GCCCAGTTCC AGTACCGCAA CCGCATCGCC 1260
1261 ATGGAGTTCA ACCAGCTGTA CCACTGGCAC CCGCTCATGC CAGACTCCTT CTGGGTGGGT 1320
1321 TCCCAGGAGT ACAGCTATGA GCAGTTCCTG TTCAACACCT CCATGCTGAC GCACTACGGG 1380
```

FIG. 9C-1

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1381 ATCGAGGCCC TGGTGGATGC CTTCTCTCGC CAGAGCGCCG GCCGGATTGG TGGAGGTAGA 1440
1441 AACATAGACC ACCATGTCCT GCACGTGGCT GTGGAAACCA TCAAGGAATC CCGCGAGTTG 1500
1501 CGGCTGCAGC CCTTCAATGA GTACCGCAAG AGGTTTGGCA TGAGGCCCTA CATGTCCTTC 1560
1561 CAGGAACTCA CAGGGGAGAA GGAGATGGCA GCCGAGTTGG AGGAGCTGTA TGGAGACATT 1620
1621 GATGCCTTGG AATTCTACCC GGGGCTTCTT CTGGAGAAGT GCCATCCAAA CTCCATCTTT 1680
1681 GGAGAGAGTA TGATAGAAAT TGGGGCTCCC TTCTCCCTTA AGGGCCTCCT AGGGAATCCC 1740
1741 ATCTGTTCTC CAGAGTACTG GAAGCCAAGC ACATTCGGTG GTGAGATGGG CTTCAATATG 1800
1801 GTCAAGACAG CCACACTGAA GAAGCTGGTC TGCCTTAACA CCAAGACTTG TCCCTATGTT 1860
1861 TCCTTCCGTG TGCCTGACCC CCACCAGGAT GGCGGGCCTG GTGTGCAGCG GCCGTCCACA 1920
1921 GAGCTCTGAG GGGGCAGAGC AGCAGCATTC TGGAGGGTGG ACTTGTCATC CCAGAATGCT 1980
1981 GAGGCTGGGG TTAATAATCC CAAATGTTGG GTCTTTGGTT TGCCTCAAGA ATATCAAGGT 2040
2041 CAACATTTAG AACTTTGTGT CTCTCACCCA TTATCTGGAA TATCATGGTC TTGTTTGTTA 2100
2101 TTCTAGAATG CTGAATTCCT GGTTGACCAT CTAGAATGGA TGGAGTGATG CTTCTTTGGC 2160
2161 AAGCCAGAAC ACTGGTTCCT GGCCGACAAC CTAGAATGTC AGACTTCTGG TTGACTTAAG 2220
2221 ACGTAGGCAT TCTCTAATGT GAAGCTCCTG ACAGAATCAT CTAGAAAGAT AGGGGATTCT 2280
2281 TATTTTGCAT TCTAGAATTC TGGGCAGCCC TCCAGCATGT TGATTTTTTT CACTGGCAGT 2340
2341 TCAGAATGTT GTGCTCTTGA TTGCTGATCC AAAATAGTGG CTGGTATGCC AGATCAGTCT 2400
2401 TGCTCTGAAT GCCTAGAATG GTAATTTGAT TCATTTTCCT GTTCAGTGAG ATACCCCCAA 2460
2461 AGCAGGAGAA TCTACAGCCT AACCAGAGTG CATTGCCTGC CTCTGTGCCT GCCCCGAGGA 2520
2521 CTTAGGGGGC AGAGTGTTCT TCCTGGGACG CTGACTCAGA CCCTGGTCCA AGGAGCTAGA 2580
2581 ACAGGTGGGC TTTTTCCAGG TCATTGGTTG GAGGCCACCA GAGCTCTGTT GCCATCTTTG 2640
2641 TCTTGACTCA TGACAGCTGT TTCTCATGAA ACTAATAAAA TTCTTTTTCC AAAAAAAAAA 2700
2701 AAAAAA                                                            2706
```

FIG. 9C-2

```
SEQ ID NO:4    1 ATGAGCCGTG AGTTCGACCC TGAGGCCCCC AGGAACCCTC TTCGCCTCCC GGGGGAGCCT   60
              61 CGAATGCCAG GCCCAGCCCT CACCTCTCGC TCCGCAGGGG GGAGTCGCCT GCACCGGTGG  120
             121 CCGCTGCTCC TGCTGCTGCT GCTGCTGCTC CCGCCGCCCC CGGTCCTGCC CGCGGAAGCC  180
             181 CGGACCCCGG CGCCTGTGAA CCCGTGTTGT TACTACCCAT GTCAGCACCA AGGGATCTGT  240
             241 GTCCGCTTCG GCCTTGACCG CTACCAGTGT GACTGCACCC GCACGGGCTA TTCTGGCCCC  300
             301 AACTGCACCA TCCCCGAGCT GTGGACCTGG CTCCGGAATT CACTGCGCCC CAGTCCCTCT  360
             361 TTCCTCCACT TCCTGCTGAC GCATGGGCGC TGGTTTTGGG AATTCATCAA TGCCACCTTC  420
             421 ATCCGTGACA TGCTCATGCG TCTGGTACTC ACAGGGGAGA CCATCAAGAT TGTGATTGAG  480
             481 GAGTATGTGC AGCAGCTGAG TGGCTACTTC TTGCAGCTGA AGTTCGACCC GGAGCTGCTG  540
             541 TTTAGCGCCC AGTTCCAGTA CCGCAACCGC ATCGCCATGG AGTTCAACCA GCTGTACCAC  600
             601 TGGCACCCGC TCATGCCAGA CTCCTTCTGG GTGGGTTCCC AGGAGTACAG CTATGAGCAG  660
             661 TTCCTGTTCA ACACCTCCAT GCTGACGCAC TACGGGATCG AGGCCCTGGT GGATGCCTTC  720
             721 TCTCGCCAGA GCGCCGGCCG GATTGGTGGA GGTAGAAACA TAGACCACCA TGTCCTGCAC  780
             781 GTGGCTGTGG AAACCATCAA GGAATCCCGC GAGTTGCGGC TGCAGCCCTT CAATGAGTAC  840
             841 CGCAAGAGGT TTGGCATGAG GCCCTACATG TCCTTCCAGG AACTCACAGG GGAGAAGGAG  900
             901 ATGGCAGCCG AGTTGGAGGA GCTGTATGGA GACATTGATG CCTTGGAATT CTACCCGGGG  960
             961 CTTCTTCTGG AGAAGTGCCA TCCAAACTCC ATCTTTGGAG AGAGTATGAT AGAAATTGGG 1020
            1021 GCTCCCTTCT CCCTTAAGGG CCTCCTAGGG AATCCCATCT GTTCTCCAGA GTACTGGAAG 1080
            1081 CCAAGCACAT TCGGTGGTGA GATGGGCTTC AATATGGTCA AGACAGCCAC ACTGAAGAAG 1140
            1141 CTGGTCTGCC TTAACACCAA GACTTGTCCC TATGTTTCCT TCCGTGTGCC TGACCCCCAC 1200
            1201 CAGGATGGCG GGCCTGGTGT GCAGCGGCCG TCCACAGAGC TCTGA                 1245
```

FIG. 9D

```
SEQ ID NO:5   1 MSREFDPEAP RNPLRLPGEP RMPGPALTSR SAGGSRLHRW PLLLLLLLLL PPPPVLPAEA  60
             61 RTPAPVNPCC YYPCQHQGIC VRFGLDRYQC DCTRTGYSGP NCTIPELWTW LRNSLRPSPS 120
            121 FLHFLLTHGR WFWEFINATF IRDMLMRLVL TGETIKIVIE EYVQQLSGYF LQLKFDPELL 180
            181 FSAQFQYRNR IAMEFNQLYH WHPLMPDSFW VGSQEYSYEQ FLFNTSMLTH YGIEALVDAF 240
            241 SRQSAGRIGG GRNIDHHVLH VAVETIKESR ELRLQPFNEY RKRFGMRPYM SFQELTGEKE 300
            301 MAAELEELYG DIDALEFYPG LLLEKCHPNS IFGESMIEIG APFSLKGLLG NPICSPEYWK 360
            361 PSTFGGEMGF NMVKTATLKK LVCLNTKTCP YVSFRVPDPH QDGGPGVQRP STELZ       415
```

FIG. 9E

```
SEQ ID NO:6   1 CGGGGAGCTC CTGGCACCGG CGCCCCGGGA  GCCCGCAGTC TGCACCCCGA GCGCAGCAGC 60
             61 CGCCCAGAGC TATGAGCCGT GAGTTCGACC  CTGAGGCCCC CAGGAACCCT CTTCGCCTCC 120
            121 CGGGGGAGCC TCGAATGCCA GGCCCAGCCC  TCACCTCTCG CTCCGCAGGG GGGAGTCGCC 180
            181 TGCACCGGTG GCCGCTGCTC CTGCTGCTGC  TGCTGCTGCT CCCGCCGCCC CCGGTCCTGC 240
            241 CCGCGGAAGC CCGGACCCCG GCGCCTGTGA  ACCCGTGTTG TTACTACCCA TGTCAGCACC 300
            301 AAGGGATCTG TGTCCGCTTC GGCCTTGACC  GCTACCAGTG TGACTGCACC CGCACGGGCT 360
            361 ATTCTGGCCC CAACTGCACC ATCCCCGAGC  TGTGGACCTG GCTCCGGAAT TCACTGCGCC 420
            421 CCAGTCCCTC TTTCCTCCAC TTCCTGCTGA  CGCATGGGCG CTGGTTTTGG GAATTCATCA 480
            481 ATGCCACCTT CATCCGTGAC ATGCTCATGC  GTCTGGTACT CACAGGGGAG ACCATCAAGA 540
            541 TTGTGATTGA GGAGTATGTG CAGCAGCTGA  GTGGCTACTT CTTGCAGCTG AAGTTCGACC 600
            601 CGGAGCTGCT GTTTAGCGCC CAGTTCCAGT  ACCGCAACCG CATCGCCATG GAGTTCAACC 660
            661 AGCTGTACCA CTGGCACCCG CTCATGCCAG  ACTCCTTCTG GGTGGGTTCC CAGGAGTACA 720
            721 GCTATGAGCA GTTCCTGTTC AACACCTCCA  TGCTGACGCA CTACGGGATC GAGGCCCTGG 780
            781 TGGATGCCTT CTCTCGCCAG AGCGCCGGCC  GGATTGGTGG AGGTAGAAAC ATAGACCACC 840
            841 ATGTCCTGCA CGTGGCTGTG GAAACCATCA  AGGAATCCCG CGAGTTGCGG CTGCAGCCCT 900
            901 TCAATGAGTA CCGCAAGAGG TTTGGCATGA  GGCCCTACAT GTCCTTCCAG GAACTCACAG 960
            961 GGGAGAAGGA GATGGCAGCC GAGTTGGAGG  AGCTGTATGG AGACATTGAT GCCTTGGAAT 1020
```

FIG. 9F-1

1021 TCTACCCGGG GCTTCTTCTG GAGAAGTGCC ATCCAAACTC CATCTTTGGA GAGAGTATGA 1080
1081 TAGAAATTGG GGCTCCCTTC TCCCTTAAGG GCCTCCTAGG GAATCCCATC TGTTCTCCAG 1140
1141 AGTACTGGAA GCCAAGCACA TTCGGTGGTG AGATGGGCTT CAATATGGTC AAGACAGCCA 1200
1201 CACTGAAGAA GCTGGTCTGC CTTAACACCA AGACTTGTCC CTATGTTTCC TTCCGTGTGC 1260
1261 CTGACCCCCA CCAGGATGGC GGGCCTGGTG TGCAGCGGCC GTCCACAGAG CTCTGAGGGG 1320
1321 GCAGAGCAGC AGCATTCTGG AGGGTGGACT TGTCATCCCA GAATGCTGAG GCTGGGGTTA 1380
1381 ATAATCCCAA ATGTTGGGTC TTTGGTTTGC CTCAAGAATA TCAAGGTCAA CATTTAGAAC 1440
1441 TTTGTGTCTC TCACCCATTA TCTGGAATAT CATGGTCTTG TTTGTTATTC TAGAATGCTG 1500
1501 AATTCCTGGT TGACCATCTA GAATGGATGG AGTGATGCTT CTTTGGCAAG CCAGAACACT 1560
1561 GGTTCCTGGC CGACAACCTA GAATGTCAGA CTTCTGGTTG ACTTAAGACG TAGGCATTCT 1620
1621 CTAATGTGAA GCTCCTGACA GAATCATCTA GAAAGATAGG GGATTCTTAT TTTGCATTCT 1680
1681 AGAATTCTGG GCAGCCCTCC AGCATGTTGA TTTTTTTCAC TGGCAGTTCA GAATGTTGTG 1740
1741 CTCTTGATTG CTGATCCAAA ATAGTGGCTG GTATGCCAGA TCAGTCTTGC TCTGAATGCC 1800
1801 TAGAATGGTA ATTTGATTCA TTTTCCTGTT CAGTGAGATA CCCCCAAAGC AGGAGAATCT 1860
1861 ACAGCCTAAC CAGAGTGCAT TGCCTGCCTC TGTGCCTGCC C 1901

FIG. 9F-2

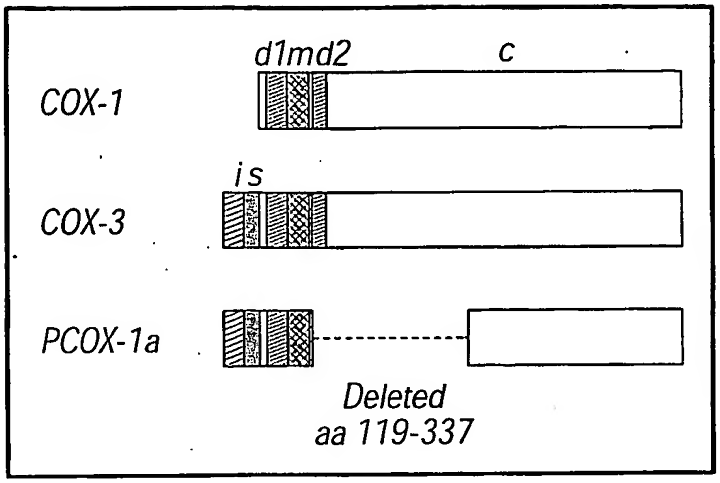
d1md2
c
COX-1
is
COX-3
PCOX-1a
Deleted
aa 119-337

FIG. 11

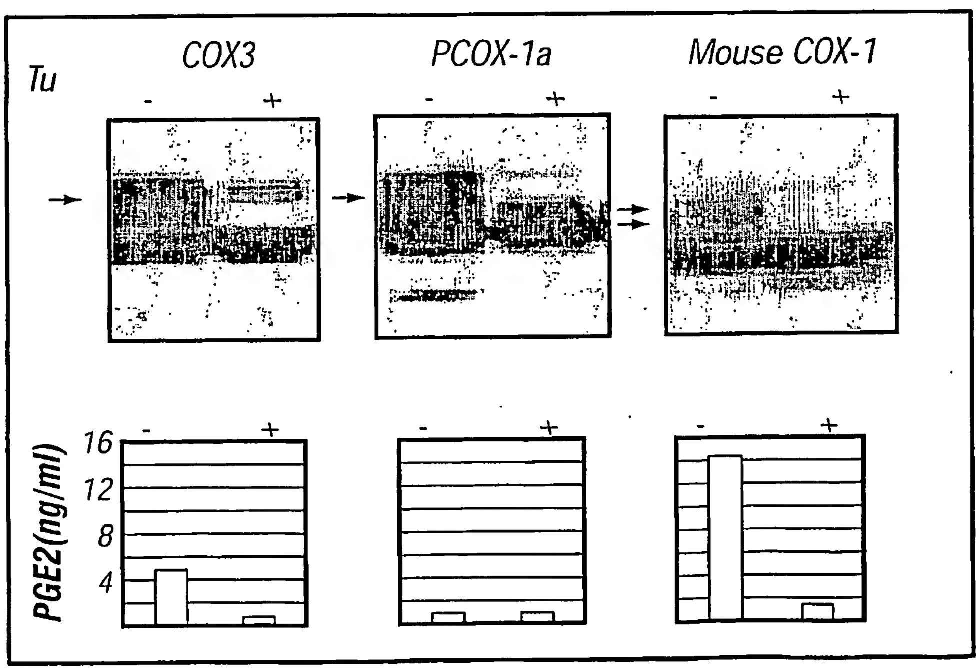
Tu
COX3
PCOX-1a
Mouse COX-1
-
+
PGE2(ng/ml)
16
12
8
4

FIG. 12

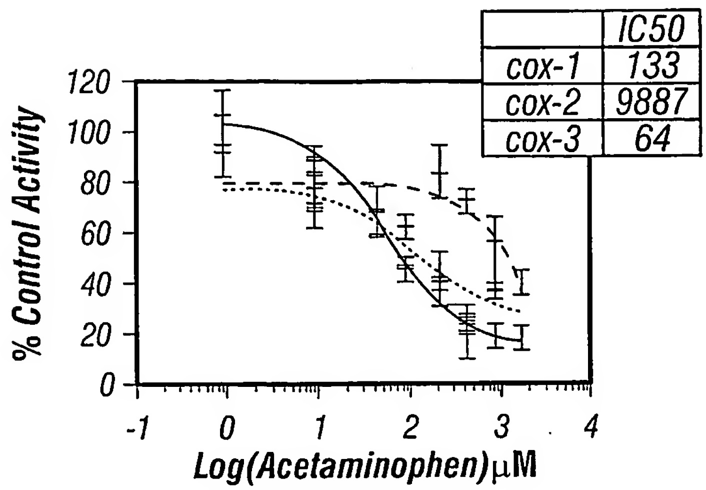

FIG. 13A

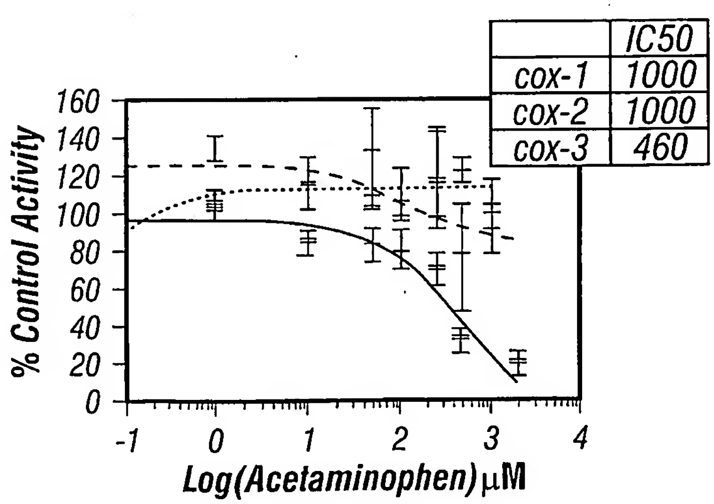

FIG. 13B

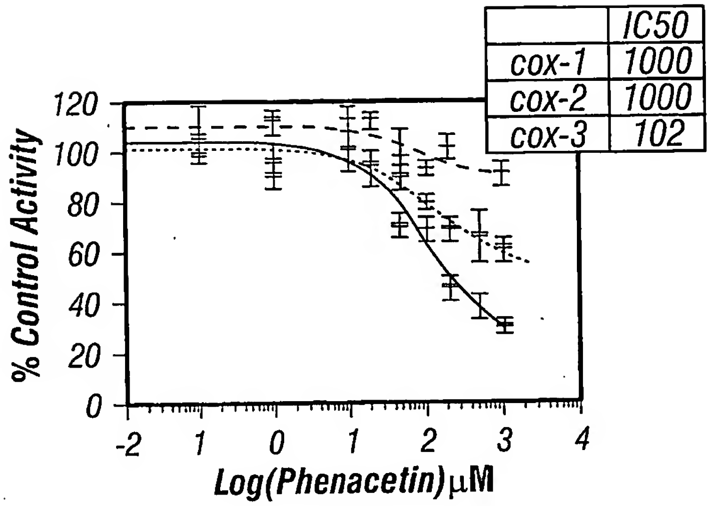

FIG. 13C

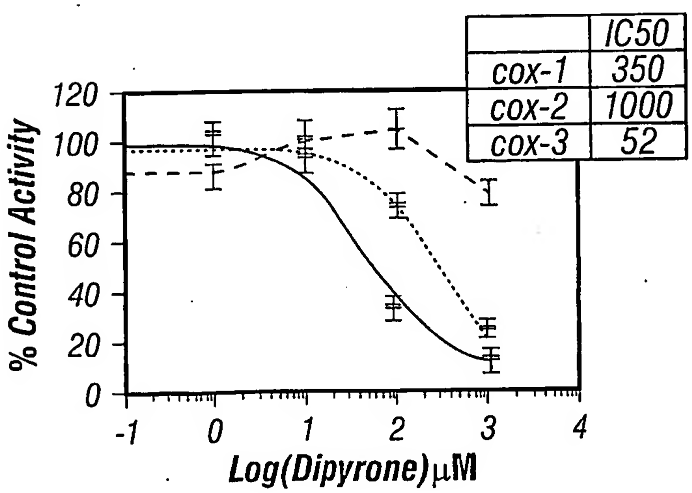

FIG. 13D

	H2	H5	H8
COX-1	FAFFAQHFTH QFFKTS	YATLWLREHN RVCD	EFNQLYHWHP LM
COX-2	FAFFAQHFTH QFFKTD	YATIWLREHN RVCD	EFNTLYHWHP LL
coral COX	FMFFAQHFTH EFFKTI	YASIWLREHN RVCT	EFNHMYHWHP FS
rice	AAAWIQFMVH DWMDHM	LQALFVKEHN AVCD	EFTSVYRMHS LI
arabidopsis	AASWIQFMIH DWIDHL	LQALFIKEHN AVCD	DFTSVYRMHS LL
tobacco	AASWIQFMIH DWIDHL	LQALFVQEHN SVCD	EFTSVYRMHQ LL
LDS	LFYLATIITH DIFQTS	FLIMFNRFHN YVVT	EFNLIYRWHC TI
consensus	.a..aq..tH d.f.t.	...lf.reHN ..Vcd	eFn..YrwH. l.

Distal Glutamine

Distal Histidine

Active Site Tyrosine

Proximal Histidine

FIG. 14

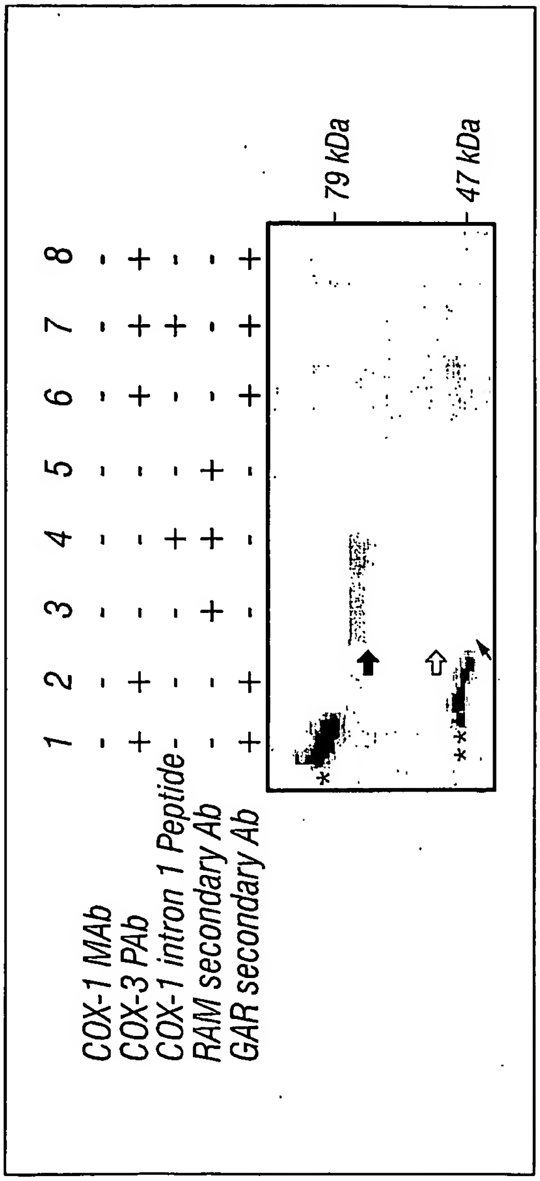

FIG. 15A

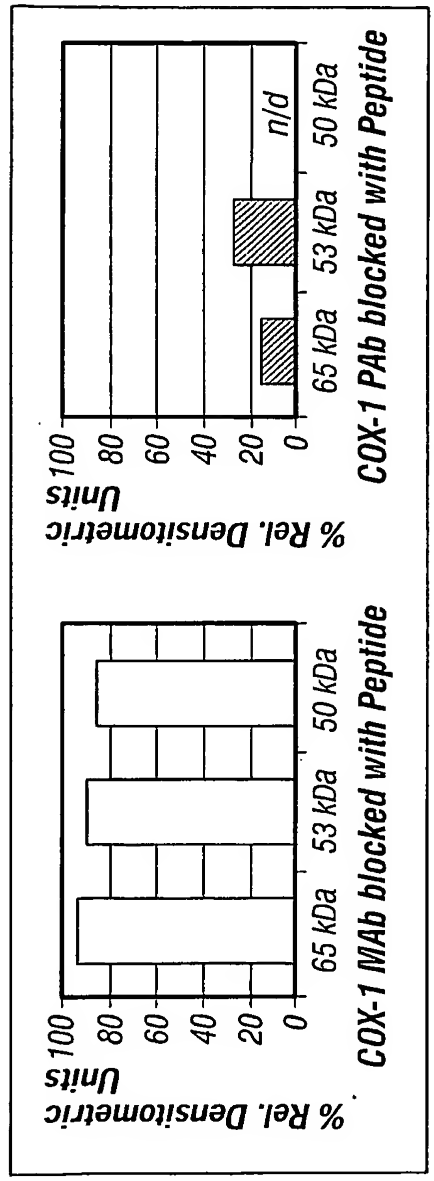

FIG. 15B

```
                1        10        20        30        40        50        60        70        80        90        100       110       120       130
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)      ATGAGCCGTGAGTGCGACCCCGGTGCCCGGTGGGGAATTTTCTTGGCCTCCTGGTGGAGCCTTGAATGCCAGGCTCAGCCCCTCATCTCTCTCCTCTGCAGGGAGTCTCTTGCTCCGGTTCTTGCTGTTC
hcox-3(af)      ATGAGCCGTGAGTGCGACCCCGGTGCCCGGTGGGGAATTTTCTTGGCCTCCTGGTGGAGCCTTGAATGCCAGGCTCAGCCCCTCATCTCTCTCCTCTGCAGGGAGTCTCTTGCTCCGGTTCTTGCTGTTC
hcox-3d(del10)  ATGAGCCGTGAGTGCGACCCCGGTGCCCGGTGGGGAATTTTCTTGGCCTCCTGGTGGAGCCTTGAATGCCAGGCTCAGCCCCTCATCTCTCTCCTCTGCAGGGAGTCTCTTGCTCCGGTTCTTGCTGTTC
hcox-3(cs)      ATGAGCCGTGAGTGCGACCCCGGTGCCCGGTGGGGAATTTTCTTGGCCTCCTGGTGGAGCCTTGAATGCCAGGCTCAGCCCCTCATCTCTCTCCTCTGCAGGGAGTCTCTTGCTCCGGTTCTTGCTGTTC

                131      140       150       160       170       180       190       200       210       220       230       240       250       260
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)      CTGCTCCTGCTCCCGCCGCTCCCCGTCCTGCTCGCGGACCCAGGGGCGCCCACGCCAGTGAATCCCTGTTGTTACTATCCATGCCAGCACCAGGGCATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGT
hcox-3(af)      CTGCTCCTGCTCCCGCCGCTCCCCGTCCTGCTCGCGGACCCAGGGGCGCCCACGCCAGTGAATCCCTGTTGTTACTATCCATGCCAGCACCAGGGCATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGT
hcox-3d(del10)  CTGCTCCTGCTCCCGCCGCTCCCCGTCCTGCTCGCGGACCCAGGGGCGCCCACGCCAGTGAATCCCTGTTGTTACTATCCATGCCAGCACCAGGGCATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGT
hcox-3(cs)      CTGCTCCTGCTCCCGCCGCTCCCCGTCCTGCTCGCGGACCCAGGGGCGCCCACGCCAGTGAATCCCTGTTGTTACTATCCATGCCAGCACCAGGGCATCTGTGTCCGCTTCGGCCTTGACCGCTACCAGT

                261      270       280       290       300       310       320       330       340       350       360       370       380       390
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)      GTGACTGCACCCGCACGGGCTATTCCGGCCCCAACTGCACCATCCCTGGCCTGTGGACCTGGCTCCGGAATTCACTGCGGCCCAGCCCCTCTTTCACCCACTTCCTGCTCACTCACGGGCGCTGGTTCTG
hcox-3(af)      GTGACTGCACCCGCACGGGCTATTCCGGCCCCAACTGCACCATCCCTGGCCTGTGGACCTGGCTCCGGAATTCACTGCGGCCCAGCCCCTCTTTCACCCACTTCCTGCTCACTCACGGGCGCTGGTTCTG
hcox-3d(del10)  GTGACTGCACCCGCACGGGCTATTCCGGCCCCAACTGCACCATCCCTGGCCTGTGGACCTGGCTCCGGAATTCGCTGCGGCCCAGCCCCTCTTTCACCCACTTCCTGCTCACTCACGGGCGCTGGTTCTG
hcox-3(cs)      GTGACTGCACCCGCACGGGCTATTCCGGCCCCAACTGCACCATCCCTGGCCTGTGGACCTGGCTCCGGAATTCaCTGCGGCCCAGCCCCTCTTTCACCCACTTCCTGCTCACTCACGGGCGCTGGTTCTG

                391      400       410       420       430       440       450       460       470       480       490       500       510       520
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)      GGAGTTTGTCAATGCCACCTTCATCCGAGAGATGCTCATGCGCCTGGTACTCACAGTGCGCTCCAACCTTATCCCCAGTCCCCCACCTACAACTCAGCACATGACTACATCAGCTGGGAGTCTTTCTCC
hcox-3(af)      GGAGTTTGTCAATGCCACCTTCATCCGAGAGATGCTCATGCGCCTGGTACTCACAGTGCGCTCCAACCTTATCCCCAGTCCCCCACCTACAACTCAGCACATGACTACATCAGCTGGGAGTCTTTCTCC
hcox-3d(del10)  GGAGTTTGTCAATGCCACCTTCATCCGAGAGATGCTCATGCGCCTGGTACTCACAGTGCGCTCCAACCTTATCCCCAGTCCCCCACCTACAACTCAGCACATGACTACATCAGCTGGGAGTCTTTCTCC
hcox-3(cs)      GGAGTTTGTCAATGCCACCTTCATCCGAGAGATGCTCATGCGCCTGGTACTCACAGTGCGCTCCAACCTTATCCCCAGTCCCCCACCTACAACTCAGCACATGACTACATCAGCTGGGAGTCTTTCTCC
```

FIG. 16A

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```
               521  530       540       550       560       570       580       590       600       610       620       630       640      650
               |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     AACGTGAGCTATTACACTCGTATTCTGCCCTCTGTGCCTAAAGATTGCCCCACACCCATGGGAACCAAAGGGAAGAAGCGGTTGCCAGATGCCCAGCTCCTGGCCCGCCGCTTCCTGCTCAGGASGAAGT
hcox-3(af)     AACGTGAGCTATTACACTCGTATTCTGCCCTCTGTGCCTAAAGATTGCCCCACACCCATGGGAACCAAAGGGAAGAAGCAGTTGCCAGATGCCCAGCTCCTGGCCCGCCGCTTCCTGCTCAGGAGGAAGT
hcox-3d(del10) AACGTGAGCTATTACACTCGTATTCTGCCCTCTGTGCCTAAAGATTGCCCCACACCCATGGGAACCAAAGGGAAGAAGCAGTTGCCAGATGCCCAGCTCCTGGCCCGCCGCTTCCTGCTCAGGAGGAAGT
hcox-3(cs)     AACGTGAGCTATTACACTCGTATTCTGCCCTCTGTGCCTAAAGATTGCCCCACACCCATGGGAACCAAAGGGAAGAAGC?GTTGCCAGATGCCCAGCTCCTGGCCCGCCGCTTCCTGCTCAGGAGGAAGT
               651  660       670       680       690       700       710       720       730       740       750       760       770      780
               |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     TCATACCTGACCCCCAAGGCACCAACCTCATGTTTGCCTTCTTTGCACAACACTTCACCCACCAGTTCTTCAAAACTTCTGGCAAGATGGGTCCTGGCTTCACCAAGGCCTTGGGCCATGGGGTGGACCT
hcox-3(af)     TCATACCTGACCCCCAAGGCACCAACCTCATGTTTGCCTTCTTTGCACAACACTTCACCCACCAGTTCTTCAAAACTTCTGGCAAGATGGGTCCTGGCTTCACCAAGGCCTTGGGCCATGGGGTAGACCT
hcox-3d(del10) TCATACCTGACCCCCAAGGCACCAACCTCATGTTTGCCTTCTTTGCACAACACTTCACCCACCAGTTCTTCAAAACTTCTGGCAAGATGGGTCCTGGCTTCACCAAGGCCTTGGGCCATGGGGTAGACCT
hcox-3(cs)     TCATACCTGACCCCCAAGGCACCAACCTCATGTTTGCCTTCTTTGCACAACACTTCACCCACCAGTTCTTCAAAACTTCTGGCAAGATGGGTCCTGGCTTCACCAAGGCCTTGGGCCATGGGGTAGACCT
               781  790       800       810       820       830       840       850       860       870       880       890       900      910
               |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     CGGCCACATTTATGGAGACAATCTGGAGCGTCAGTATCAACTGCGGCTCTTTAAGGATGGGAAACTCAAGTACCAGGTGCTGGATGGAGAAATGTACCCGCCCTCGGTAGAAGAGGCGCCTGTGTTGATG
hcox-3(af)     CGGCCACATTTATGGAGACAATCTGGAGCGTCAGTATCAACTGCGGCTCTTTAASGATGGGAAACTCAAGTACCAGGTGCTGGATGGAGAAATGTGCCCGCCCTCGGTAGAAGAGGCGCCTGTGTTGATG
hcox-3d(del10) CGGCCACATTTATGGAGACAATCTGGAGCGTCAGTATCAACTGCGGCTCTTTAAGGATGGGAAACTCAAGTACCAGGTGCTGGATGGAGAAATGTACCCGCCCTCGGTAGAGGAGGCGCCTGTGTTGATG
hcox-3(cs)     CGGCCACATTTATGGAGACAATCTGGAGCGTCAGTATCAACTGCGGCTCTTTAAGGATGGGAAACTCAAGTACCAGGTGCTGGATGGAGAAATGT?CCCGCCCTCGGTAGAAGAGGCGCCTGTGTTGATG
               911  920       930       940       950       960       970       980       990      1000      1010      1020      1030     1040
               |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     CACTACCCCCGAGGCATCCCGCCCCAGAGCCAGATGGCTGTGGGCCAGGAGGTGTTTGGGCTGCTTCCTGGGCTCATGCTGTATGCCACGCTCTGGCTACGTGAGCACAACCGTGTGTGTGACCTGCTGA
hcox-3(af)     CACTACCCCCGAGGCATCCCGCCCCAGAGCCAGATGGCTGTGGGCCAGGAGGTGTTTGGGCTGCTTCCTGGGCTCATGCTGTATGCCACGCTCTGGCTACGTGAGCACAACCGTGTGTGTGACCTGCTGA
hcox-3d(del10) CACTACCCCCGAGGCATCCCGCCCCAGAGCCAGATGGCTGTGGGCCAGGAGGTGTTTGGGCTGCTTCCTGGGCTCATGCTGTATGCCACGCTCTGGCTACGTGAGCACAACCGTGTGTGTGACCTGCTGA
hcox-3(cs)     CACTACCCCCGAGGCATCCCGCCCCAGAGCCAGATGGCTGTGGGCCAGGAGGTGTTTGGGCTGCTTCCTGGGCTCATGCTGTATGCCACGCTCTGGCTACGTGAGCACAACCGTGTGTGTGACCTGCTGA
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FIG. 16B

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               1041      1050      1060      1070      1000      1050      1100      1110      1120      1130      1140      1150      1160      1170
               |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     AGGCTGAGCACCCCACCTGGGGCGATGAGCAGCTTTTCCAGACGACCCGCCTCATCCTCATAGGGGAGACCATCAAGATTGTCATCGAGGAGTACGTGCAGCAGCTGAGTGGCTATTTCCTGCAGCTGAA
hcox-3(af)     AGGCTGAGCACCCCACCTGGGGCGATGAGCAGCTTTTCCAGACGACCCGCCTCATCCTCATAGGGGAGACCATCAAGATTGTCATCGAGGAGTACGTGCAGCAGCTGAGTGGCTATTTCCTGCAGCTGAA
hcox-3d(del10) AGGCTGAGCACCCCACCTGGGGGGATGAGCAGCTTTTCCAGACGACCCGCCTCATCCTCATAGGGGAGACCATCAAGATTGTCATCGAGGAGTACGTGCAGCAGCTGAGTGGCTATTTCCTGCAGCTGAA
hcox-3(cs)     AGGCTGAGCACCCCACCTGGGGGGATGAGCAGCTTTTCCAGACGACCCGCCTCATCCTCATAGGGGAGACCATCAAGATTGTCATCGAGGAGTACGTGCAGCAGCTGAGTGGCTATTTCCTGCAGCTGAA

               1171      1180      1190      1200      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
               |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     ATTTGACCCAGAGCTGCTGTTCGGTGTCCAGTTCCAATACCGCAACCGCATTGCCATGGAGTTCAACCATCTCTACCACTGGCACCCCCTCATGCCTGACTCCTTCAAGGTGGGCTCCCAGGAGTACAGC
hcox-3(af)     ATTTGACCCAGAGCTGCTGTTCGGTGTCCAGTTCCAATACCGCAACCGCATTGCCATGGAGTTCAACCATCTCTACCACTGGCACCCCCTCATGCCTGACTCCTTCAAGGTGGGCTCCCAGGAGTACAGC
hcox-3d(del10) ATTTGACCCAGAGCTGCTGTTCGGTGTCCTGTTCCAATACTGCAACCGCATTGCCATGGAGTTCAACCATCTCTACCACTGGCACCCCCTCATGCCTGACTCCTTCAAGGTGGGCTCCCAGGAGTACAGC
hcox-3(cs)     ATTTGACCCAGAGCTGCTGTTCGGTGTCC?GTTCCAATACCGCAACCGCATTGCCATGGAGTTCAACCATCTCTACCACTGGCACCCCCTCATGCCTGACTCCTTCAAGGTGGGCTCCCAGGAGTACAGC

               1301      1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430
               |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     TACGAGCAGTTCTTGTTCAACACCTCCATGTTGGTGGACTATGGGGTTGAGGCCCTGGTGGATGCCTTCTCTCGCCAGATTGCTGGCCGGATCGGTGGGGGCAGGAACATGGACCACCACATCCTGCATG
hcox-3(af)     TACGAGCAGTTCTTGTTCAACACCTCCATGTTGGTGGACTATGGGGTTGAGGCCCTGGTGGATGCCTTCTCTCGCCAGATTGCTGGCCGGATCGGTGGGGGCAGGAACATGGACCACCACATCCTGCATG
hcox-3d(del10) TACGAGCAGTTCTTGTTCAACACCTCCATGTTAGTGGACTATGGGGTTGAGGCCCTGGTGGATGCCTTCTCTCGCCAGATTACTGGCCGG----------------------------------------
hcox-3(cs)     TACGAGCAGTTCTTGTTCAACACCTCCATGTTgGTGGACTATGGGGTTGAGGCCCTGGTGGATGCCTTCTCTCGCCAGATTgCTGGCCGGatcggtgggggcaggaacatggaccaccacatcctgcatg

               1431      1440      1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560
               |---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)     TGGCTGTGGATGTCATCAGGGAGTCTCGGGAGATGCGGCTGCAGCCCTTCAATGAGTACCGCAAGAGGTTTGGCATGAAACCCTACACCTCCTTCCAGGAGCTCGTAGGAGAGAAGGAGATGGCAGCAGA
hcox-3(af)     TGGCTGTGGATGTCATCAGGGAGTCTCGGGAGATGCGGCTGCAGCCCTTCAATGAGTACCGCAAGAGGTTTGGCATGAAACCCTACACCTCCTTCCAGGAGCTCGTAGGAGAGAAGGAGATGGCAGCAGA
hcox-3d(del10) ---------------------------------------------------------------------------------------------------------------GAGAGAAGGAGATGGCAGCAGA
hcox-3(cs)     tggctgtggatgtcatcagggagtctcgggagatgcggctgcagcccttcaatgagtaccgcaagaggtttggcatgaaaccctacacctccttccaggagctcgtagGAGAGAAGGAGATGGCAGCAGA
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FIG. 16C

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              1561     1570      1580      1590      1600      1610      1620      1630      1640      1650      1660      1670      1680      1690
              |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)    GTTGGAGGAATCGTATGGAGACATTGATGCGTTGGAGTTCTACCCTGGACTGCTTCTTGAAAAGTGCCATCCAAACTCTATCTTTGGGGAGAGTATGATAGAGATTGGGGCTCCCTTTTCCCTCAAGGGT
hcox-3(af)    GTTGGAGGAATTGTATGGAGACATTGATGCGTTGGAGTTCTACCCTGGACTGCTTCTTGAAAAGTGCCATCCAAACTCTATCTTTGGGGAGAGTATGATAGAGATTGGGGCTCCCTTTTCCCTCAAGGGT
hcox-3d(del10) GTTGGAGGAATTGTATGGAGACATTGATGCGTTGGAGTTCTACCCTGGACTGCTTCTTGAAAAGTGCCATCCAAACTCTATCTTTGGGGAGAGTATGATAGAGATTGGGGCTCCCTTTTCCCTCAAGGGT
hcox-3(cs)    GTTGGAGGAATtGTATGGAGACATTGATGCGTTGGAGTTCTACCCTGGACTGCTTCTTGAAAAGTGCCATCCAAACTCTATCTTTGGGGAGAGTATGATAGAGATTGGGGCTCCCTTTTCCCTCAAGGGT
              1691     1700      1710      1720      1730      1740      1750      1760      1770      1780      1790      1800      1810      1820
              |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)    CTCCTAGGGAATCCCATCTGTTCTCCGGAGTACTGGAAGCCGAGCACATTTGGCGGCGAGGTGGGCTTTAACATTGTCAAGACGGCCACACTGAAGAAGCTGGTCTGCCTCAACACCAAGACCTGTCCCT
hcox-3(af)    CTCCTAGGGAATCCCATCTGTTCTCCGGAGTACTGGAAGCCGAGCACATTTGGCGGCGAGGTGGGCTTTAACATTGTCAAGACGGCCACACTGAAGAAGCTGGTCTGCCTCAACACCAAGACCTGTCCCT
hcox-3d(del10) CTCCTAGGGAATCCCATCTGTTCTCCGGAGTACTGGAAGCCGAGCACATTTGGCGGCGAGGTGGGCTTTAACATTGTCAAGACGGCCACACTGAAGAAGCTGGTCTGCCTCAACACCAAGACCTGTCCCT
hcox-3(cs)    CTCCTAGGGAATCCCATCTGTTCTCCGGAGTACTGGAAGCCGAGCACATTTGGCGGCGAGGTGGGCTTTAACATTGTCAAGACGGCCACACTGAAGAAGCTGGTCTGCCTCAACACCAAGACCTgTCCCT
              1821     1830      1840      1850      1860      1870      1880      1890   1894
              |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
hcox-3(cc)    ACGTTTCCTTCCGTGTGCCGGATGCCAGTCAGGATGATGGGCCTGCTGTGGAGCGACCATCCACAGAGCTCTGA
hcox-3(af)    ACGTTCCCTTCCGTGTGCCGGATGCCAGTCAGGATGATGGGCCTGCTGTGGAGCGACCATCCACAGAGCTCTGA
hcox-3d(del10) ACGTTTCCTTCCGTGTGCCGGATGCCAGTCAGGATGATGGGCCTGCTGTGGAGCGACCATCCACAGAGCTCTGA
hcox-3(cs)    ACGTTCCCTTCCGTGTGCCGGATGCCAGTCAGGATGATGGGCCTGCTGTGGAGCGACCATCCACAGAGCTCTGA
```

FIG. 16D

hCOX-3(cc)

```
SEQ ID NO:10  1 atgagccgtg agtgcgaccc cggtgcccgg tggggaattt tcttggcctc ctggtggagc 60
             61 cttgaatgcc aggctcagcc cctcatctct ctcctctgca gggagtctct tgctccggtt 120
            121 cttgctgttc ctgctcctgc tcccgccgct ccccgtcctg ctcgcggacc caggggcgcc 180
            181 cacgccagtg aatccctgtt gttactatcc atgccagcac cagggcatct gtgtccgctt 240
            241 cggccttgac cgctaccagt gtgactgcac ccgcacgggc tattccggcc ccaactgcac 300
            301 catccctggc ctgtggacct ggctccggaa ttcactgcgg cccagcccct ctttcaccca 360
            361 cttcctgctc actcacgggc gctggttctg ggagtttgtc aatgccacct tcatccgaga 420
            421 gatgctcatg cgcctggtac tcacagtgcg ctccaacctt atccccagtc cccccaccta 480
            481 caactcagca catgactaca tcagctggga gtctttctcc aacgtgagct attacactcg 540
            541 tattctgccc tctgtgccta aagattgccc cacacccatg ggaaccaaag ggaagaagcg 600
            601 gttgccagat gcccagctcc tggcccgccg cttcctgctc aggaggaagt tcatacctga 660
            661 cccccaaggc accaacctca tgtttgcctt ctttgcacaa cacttcaccc accagttctt 720
            721 caaaacttct ggcaagatgg gtcctggctt caccaaggcc ttgggccatg ggtagacct 780
            781 cggccacatt tatggagaca atctggagcg tcagtatcaa ctgcggctct ttaaggatgg 840
            841 gaaactcaag taccaggtgc tggatggaga aatgtacccg ccctcggtag aagaggcgcc 900
            901 tgtgttgatg cactaccccc gaggcatccc gccccagagc cagatggctg tgggccagga 960
            961 ggtgtttggg ctgcttcctg ggctcatgct gtatgccacg ctctggctac gtgagcacaa 1020
           1021 ccgtgtgtgt gacctgctga aggctgagca ccccacctgg ggcgatgagc agcttttcca 1080
           1081 gacgacccgc ctcatcctca taggggagac catcaagatt gtcatcgagg agtacgtgca 1140
           1141 gcagctgagt ggctatttcc tgcagctgaa atttgaccca gagctgctgt tcggtgtcca 1200
```

FIG. 17A

1201 gttccaatac cgcaaccgca ttgccatgga gttcaaccat ctctaccact ggcaccccct 1260
1261 catgcctgac tccttcaagg tgggctccca ggagtacagc tacgagcagt tcttgttcaa 1320
1321 cacctccatg ttggtggact atggggttga ggccctggtg gatgccttct ctcgccagat 1380
1381 tgctggccgg atcggtgggg gcaggaacat ggaccaccac atcctgcatg tggctgtgga 1440
1441 tgtcatcagg gagtctcggg agatgcggct gcagcccttc aatgagtacc gcaagaggtt 1500
1501 tggcatgaaa ccctacacct ccttccagga gctcgtagga gagaaggaga tggcagcaga 1560
1561 gttggaggaa ttgtatggag acattgatgc gttggagttc taccctggac tgcttcttga 1620
1621 aaagtgccat ccaaactcta tctttgggga gagtatgata gagattgggg ctcccttttc 1680
1681 cctcaagggt ctcctaggga atcccatctg ttctccggag tactggaagc cgagcacatt 1740
1741 tggcggcgag gtgggcttta acattgtcaa gacggccaca ctgaagaagc tggtctgcct 1800
1801 caacaccaag acctgtccct acgtttcctt ccgtgtgccg gatgccagtc aggatgatgg 1860
1861 gcctgctgtg gagcgaccat ccacagagct ctga 1894

FIG. 17B

hCOX-3(af)

```
SEQ ID NO:11    1 ATGAGCCGTG AGTGCGACCC CGGTGCCCGG TGGGGAATTT TCTTGGCCTC CTGGTGGAGC 60
               61 CTTGAATGCC AGGCTCAGCC CCTCATCTCT CTCCTCTGCA GGGAGTCTCT TGCTCCGGTT 120
              121 CTTGCTGTTC CTGCTCCTGC TCCCGCCGCT CCCCGTCCTG CTCGCGGACC CAGGGGCGCC 180
              181 CACGCCAGTG AATCCCTGTT GTTACTATCC ATGCCAGCAC CAGGGCATCT GTGTCCGCTT 240
              241 CGGCCTTGAC CGCTACCAGT GTGACTGCAC CCGCACGGGC TATTCCGGCC CCAACTGCAC 300
              301 CATCCCTGGC CTGTGGACCT GGCTCCGGAA TTCACTGCGG CCCAGCCCCT CTTTCACCCA 360
              361 CTTCCTGCTC ACTCACGGGC GCTGGTTCTG GGAGTTTGTC AATGCCACCT TCATCCGAGA 420
              421 GATGCTCATG CGCCTGGTAC TCACAGTGCG CTCCAACCTT ATCCCCAGTC CCCCCACCTA 480
              481 CAACTCAGCA CATGACTACA TCAGCTGGGA GTCTTTCTCC AACGTGAGCT ATTACACTCG 540
              541 TATTCTGCCC TCTGTGCCTA AAGATTGCCC CACACCCATG GGAACCAAAG GGAAGAAGCA 600
              601 GTTGCCAGAT GCCCAGCTCC TGGCCCGCCG CTTCCTGCTC AGGAGGAAGT TCATACCTGA 660
              661 CCCCCAAGGC ACCAACCTCA TGTTTGCCTT CTTTGCACAA CACTTCACCC ACCAGTTCTT 720
              721 CAAAACTTCT GGCAAGATGG GTCCTGGCTT CACCAAGGCC TTGGGCCATG GGGTAGACCT 780
              781 CGGCCACATT TATGGAGACA ATCTGGAGCG TCAGTATCAA CTGCGGCTCT TTAAGGATGG 840
              841 GAAACTCAAG TACCAGGTGC TGGATGGAGA AATGTGCCCG CCCTCGGTAG AAGAGGCGCC 900
              901 TGTGTTGATG CACTACCCCC GAGGCATCCC GCCCCAGAGC CAGATGGCTG TGGGCCAGGA 960
              961 GGTGTTTGGG CTGCTTCCTG GGCTCATGCT GTATGCCACG CTCTGGCTAC GTGAGCACAA 1020
             1021 CCGTGTGTGT GACCTGCTGA AGGCTGAGCA CCCCACCTGG GGCGATGAGC AGCTTTTCCA 1080
             1081 GACGACCCGC CTCATCCTCA TAGGGGAGAC CATCAAGATT GTCATCGAGG AGTACGTGCA 1140
             1141 GCAGCTGAGT GGCTATTTCC TGCAGCTGAA ATTTGACCCA GAGCTGCTGT TCGGTGTCCA 1200
```

FIG. 18A

```
1201 GTTCCAATAC CGCAACCGCA TTGCCATGGA GTTCAACCAT CTCTACCACT GGCACCCCCT 1260
1261 CATGCCTGAC TCCTTCAAGG TGGGCTCCCA GGAGTACAGC TACGAGCAGT TCTTGTTCAA 1320
1321 CACCTCCATG TTGGTGGACT ATGGGGTTGA GGCCCTGGTG GATGCCTTCT CTCGCCAGAT 1380
1381 TGCTGGCCGG ATCGGTGGGG GCAGGAACAT GGACCACCAC ATCCTGCATG TGGCTGTGGA 1440
1441 TGTCATCAGG GAGTCTCGGG AGATGCGGCT GCAGCCCTTC AATGAGTACC GCAAGAGGTT 1500
1501 TGGCATGAAA CCCTACACCT CCTTCCAGGA GCTCGTAGGA GAGAAGGAGA TGGCAGCAGA 1560
1561 GTTGGAGGAA TTGTATGGAG ACATTGATGC GTTGGAGTTC TACCCTGGAC TGCTTCTTGA 1620
1621 AAAGTGCCAT CCAAACTCTA TCTTTGGGGA GAGTATGATA GAGATTGGGG CTCCCTTTTC 1680
1681 CCTCAAGGGT CTCCTAGGGA ATCCCATCTG TTCTCCGGAG TACTGGAAGC CGAGCACATT 1740
1741 TGGCGGCGAG GTGGGCTTTA ACATTGTCAA GACGGCCACA CTGAAGAAGC TGGTCTGCCT 1800
1801 CAACACCAAG ACCTGTCCCT ACGTTCCCTT CCGTGTGCCG GATGCCAGTC AGGATGATGG 1860
1861 GCCTGCTGTG GAGCGACCAT CCACAGAGCT CTGA                             1894
```

FIG. 18B

hCOX-3(del10)

```
SEQ ID NO:12  1 ATGAGCCGTG AGTGCGACCC CGGTGCCCGG TGGGGAATTT TCTTGGCCTC CTGGTGGAGC 60
             61 CTTGAATGCC AGGCTCAGCC CCTCATCTCT CTCCTCTGCA GGGAGTCTCT TGCTCCGGTT 120
            121 CTTGCTGTTC CTGCTCCTGC TCCCGCCGCT CCCCGTCCTG CTCGCGGACC CAGGGGCGCC 180
            181 CACGCCAGTG AATCCCTGTT GTTACTATCC ATGCCAGCAC CAGGGCATCT GTGTCCGCTT 240
            241 CGGCCTTGAC CGCTACCAGT GTGACTGCAC CCGCACGGGC TATTCCGGCC CCAACTGCAC 300
            301 CATCCCTGGC CTGTGGACCT GGCTCCGGAA TTCGCTGCGG CCCAGCCCCT CTTTCACCCA 360
            361 CTTCCTGCTC ACTCACGGGC GCTGGTTCTG GGAGTTTGTC AATGCCACCT TCATCCGAGA 420
            421 GATGCTCATG CGCCTGGTAC TCACAGTGCG CTCCAACCTT ATCCCCAGTC CCCCCACCTA 480
            481 CAACTCAGCA CATGACTACA TCAGCTGGGA GTCTTTCTCC AACGTGAGCT ATTACACTCG 540
            541 TATTCTGCCC TCTGTGCCTA AAGATTGCCC CACACCCATG GGAACCAAAG GGAAGAAGCA 600
            601 GTTGCCAGAT GCCCAGCTCC TGGCCCGCCG CTTCCTGCTC AGGAGGAAGT TCATACCTGA 660
            661 CCCCCAAGGC ACCAACCTCA TGTTTGCCTT CTTTGCACAA CACTTCACCC ACCAGTTCTT 720
            721 CAAAACTTCT GGCAAGATGG GTCCTGGCTT CACCAAGGCC TTGGGCCATG GGGTAGACCT 780
            781 CGGCCACATT TATGGAGACA ATCTGGAGCG TCAGTATCAA CTGCGGCTCT TTAAGGATGG 840
            841 GAAACTCAAG TACCAGGTGC TGGATGGAGA AATGTACCCG CCCTCGGTAG AGGAGGCGCC 900
            901 TGTGTTGATG CACTACCCCC GAGGCATCCC GCCCCAGAGC CAGATGGCTG TGGGCCAGGA 960
            961 GGTGTTTGGG CTGCTTCCTG GGCTCATGCT GTATGCCACG CTCTGGCTAC GTGAGCACAA 1020
           1021 CCGTGTGTGT GACCTGCTGA AGGCTGAGCA CCCCACCTGG GGGGATGAGC AGCTTTTCCA 1080
           1081 GACGACCCGC CTCATCCTCA TAGGGGAGAC CATCAAGATT GTCATCGAGG AGTACGTGCA 1140
           1141 GCAGCTGAGT GGCTATTTCC TGCAGCTGAA ATTTGACCCA GAGCTGCTGT TCGGTGTCCT 1200
```

FIG. 19A

```
1201 GTTCCAATAC TGCAACCGCA TTGCCATGGA GTTCAACCAT CTCTACCACT GGCACCCCCT 1260
1261 CATGCCTGAC TCCTTCAAGG TGGGCTCCCA GGAGTACAGC TACGAGCAGT TCTTGTTCAA 1320
1321 CACCTCCATG TTAGTGGACT ATGGGGTTGA GGCCCTGGTG GATGCCTTCT CTCGCCAGAT 1380
1381 TACTGGCCGG GAGAGAAGGA GATGGCAGCA GAGTTGGAGG AATTGTATGG AGACATTGAT 1440
1441 GCGTTGGAGT TCTACCCTGG ACTGCTTCTT GAAAAGTGCC ATCCAAACTC TATCTTTGGG 1500
1501 GAGAGTATGA TAGAGATTGG GGCTCCCTTT TCCCTCAAGG GTCTCCTAGG GAATCCCATC 1560
1561 TGTTCTCCGG AGTACTGGAA GCCGAGCACA TTTGGCGGCG AGGTGGGCTT TAACATTGTC 1620
1621 AAGACGGCCA CACTGAAGAA GCTGGTCTGC CTCAACACCA AGACCTGTCC CTACGTTTCC 1680
1681 TTCCGTGTGC CGGATGCCAG TCAGGATGAT GGGCCTGCTG TGGAGCGACC ATCCACAGAG 1740
1741 CTCTGA                                                           1746
```

FIG. 19B

hCOX-3(con-p)

```
  1  ATGAGCCGTG AGTGCGACCC CGGTGCCCGG TGGGGAATTT SEQ ID NO:13
     M  S  R  E  C  D  P  G  A  R  W  G  I  F   SEQ ID NO:14
      .  A  V  S  A  T  P  V  P  G  G  E  F     SEQ ID NO:15
      E  P  .  V  R  P  R  C  P  V  G  N  F     SEQ ID NO:16
 41  TCTTGGCCTC CTGGTGGAGC CTTGAATGCC AGGCTCAGCC
        L  A  S  W  W  S  L  E  C  Q  A  Q  P
     S  W  P  P  G  G  A  L  N  A  R  L  S  P
      L  G  L  L  V  E  P  .  M  P  G  S  A
 81  CCTCATCTCT CTCCTCTGCA GGGAGTCTCT TGCTCCGGTT
      L  I  S  L  L  C  R  E  S  L  A  P  V
       S  S  L  S  S  A  G  S  L  L  L  R  F
     P  H  L  S  P  L  Q  G  V  S  C  S  G  S
121  CTTGCTGTTC CTGCTCCTGC TCCCGCCGCT CCCCGTCCTG
     L  A  V  P  A  P  A  P  A  A  P  R  P  A
      L  L  F  L  L  L  L  P  P  L  P  V  L
       C  C  S  C  S  C  S  R  R  S  P  S  C
161  CTCGCGGACC CAGGGGCGCC CACGCCAGTG AATCCCTGTT
       R  G  P  R  G  A  H  A  S  E  S  L  L
     L  A  D  P  G  A  P  T  P  V  N  P  C  C
      S  R  T  Q  G  R  P  R  Q  .  I  P  V
201  GTTACTATCC ATGCCAGCAC CAGGGCATCT GTGTCCGCTT
      L  L  S  M  P  A  P  G  H  L  C  P  L
       Y  Y  P  C  Q  H  Q  G  I  C  V  R  F
     V  T  I  H  A  S  T  R  A  S  V  S  A  S
241  CGGCCTTGAC CGCTACCAGT GTGACTGCAC CCGCACGGGC
     R  P  .  P  L  P  V  .  L  H  P  H  G  L
      G  L  D  R  Y  Q  C  D  C  T  R  T  G
       A  L  T  A  T  S  V  T  A  P  A  R  A
281  TATTCCGGCC CCAACTGCAC CATCCCTGGC CTGTGGACCT
       F  R  P  Q  L  H  H  P  W  P  V  D  L
     Y  S  G  P  N  C  T  I  P  G  L  W  T  W
      I  P  A  P  T  A  P  S  L  A  C  G  P
321  GGCTCCGGAA TTCACTGCGG CCCAGCCCCT CTTTCACCCA
      A  P  E  F  T  A  A  Q  P  L  F  H  P
       L  R  N  S  L  R  P  S  P  S  F  T  H
     G  S  G  I  H  C  G  P  A  P  L  S  P  T
```

FIG. 20A

hCOX-3(con-p)

```
361  CTTCCTGCTC ACTCACGGGC GCTGGTTCTG GGAGTTTGTC
     L  P  A  H   S  R  A   L  V  L   G  V  C  Q
      F  L  L   T  H  G  R   W  F  W   E  F  V
       S  C  S   L  T  G   A  G  S  G   S  L  S
401  AATGCCACCT TCATCCGAGA GATGCTCATG CGCCTGGTAC
       C  H  L   H  P  R   D  A  H  A   P  G  T
     N  A  T  F   I  R  E   M  L  M   R  L  V  L
      M  P  P   S  S  E  R   C  S  C   A  W  Y
441  TCACAGTGCG CTCCAACCTT ATCCCCAGTC CCCCCACCTA
      H  S  A   L  Q  P  Y   P  Q  S   P  H  L
       T  V  R   S  N  L   I  P  S  P   P  T  Y
     S  Q  C  A   P  T  L   S  P  V   P  P  P  T
481  CAACTCAGCA CATGACTACA TCAGCTGGGA GTCTTTCTCC
     Q  L  S  T   .  L  H   Q  L  G   V  F  L  Q
      N  S  A   H  D  Y  I   S  W  E   S  F  S
       T  Q  H   M  T  T   S  A  G  S   L  S  P
521  AACGTGAGCT ATTACACTCG TATTCTGCCC TCTGTGCCTA
       R  E  L   L  H  S   Y  S  A  L   C  A  .
     N  V  S  Y   Y  T  R   I  L  P   S  V  P  K
      T  .  A   I  T  L  V   F  C  P   L  C  L
561  AAGATTGCCC CACACCCATG GGAACCAAAG GGAAGAAGCA
      R  L  P   H  T  H  G   N  Q  R   E  E  A
       D  C  P   T  P  M   G  T  K  G   K  K  Q
     K  I  A  P   H  P  W   E  P  K   G  R  S  S
601  GTTGCCAGAT GCCCAGCTCC TGGCCCGCCG CTTCCTGCTC
     V  A  R  C   P  A  P   G  P  P   L  P  A  Q
      L  P  D   A  Q  L  L   A  R  R   F  L  L
       C  Q  M   P  S  S   W  P  A  A   S  C  S
641  AGGAGGAAGT TCATACCTGA CCCCCAAGGC ACCAACCTCA
       E  E  V   H  T  .   P  P  R  H   Q  P  H
     R  R  K  F   I  P  D   P  Q  G   T  N  L  M
      G  G  S   S  Y  L  T   P  K  A   P  T  S
681  TGTTTGCCTT CTTTGCACAA CACTTCACCC ACCAGTTCTT
      V  C  L   L  C  T  T   L  H  P   P  V  L
       F  A  F   F  A  Q   H  F  T  H   Q  F  F
     C  L  P  S   L  H  N   T  S  P   T  S  S  S
```

FIG. 20B

hCOX-3(con-p)

```
721   CAAAACTTCT GGCAAGATGG GTCCTGGCTT CACCAAGGCC
      Q  N  F  W   Q  D  G   S  W  L   H  Q  G  L
       K  T  S   G  K  M  G   P  G  F   T  K  A
        K  L  L   A  R  W   V  L  A  S   P  R  P
761   TTGGGCCATG GGGTAGACCT CGGCCACATT TATGGAGACA
        G  P  W   G  R  P   R  P  H  L   W  R  Q
      L  G  H  G   V  D  L   G  H  I   Y  G  D  N
       W  A  M   G  .  T  S   A  T  F   M  E  T
801   ATCTGGAGCG TCAGTATCAA CTGCGGCTCT TTAAGGATGG
       S  G  A   S  V  S  T   A  A  L   .  G  W
        L  E  R   Q  Y  Q   L  R  L  F   K  D  G
      I  W  S  V   S  I  N   C  G  S   L  R  M  G
841   GAAACTCAAG TACCAGGTGC TGGATGGAGA AATGTACCCG
      E  T  Q  V   P  G  A   G  W  R   N  V  P  A
       K  L  K   Y  Q  V  L   D  G  E   M  Y  P
        N  S  S   T  R  C   W  M  E  K   C  T  R
881   CCCTCGGTAG AAGAGGCGCC TGTGTTGATG CACTACCCCC
        L  G  R   R  G  A   C  V  D  A   L  P  P
      P  S  V  E   E  A  P   V  L  M   H  Y  P  R
       P  R  .   K  R  R  L   C  .  C   T  T  P
921   GAGGCATCCC GCCCCAGAGC CAGATGGCTG TGGGCCAGGA
       R  H  P   A  P  E  P   D  G  C   G  P  G
        G  I  P   P  Q  S   Q  M  A  V   G  Q  E
      E  A  S  R   P  R  A   R  W  L   W  A  R  R
961   GGTGTTTGGG CTGCTTCCTG GGCTCATGCT GTATGCCACG
      G  V  W  A   A  S  W   A  H  A   V  C  H  A
       V  F  G   L  L  P  G   L  M  L   Y  A  T
        C  L  G   C  F  L   G  S  C  C   M  P  R
1001  CTCTGGCTAC GTGAGCACAA CCGTGTGTGT GACCTGCTGA
        L  A  T   .  A  Q   P  C  V  .   P  A  E
      L  W  L  R   E  H  N   R  V  C   D  L  L  K
       S  G  Y   V  S  T  T   V  C  V   T  C  .
1041  AGGCTGAGCA CCCCACCTGG GGCGATGAGC AGCTTTTCCA
       G  .  A   P  H  L  G   R  .  A   A  F  P
        A  E  H   P  T  W   G  D  E  Q   L  F  Q
      R  L  S  T   P  P  G   A  M  S   S  F  S  R
```

FIG. 20C

hCOX-3(con-p)

```
1081  GACGACCCGC CTCATCCTCA TAGGGGAGAC CATCAAGATT
      D  D  P  P   H  P  H   R  G  D   H  Q  D  C
       T  T  R   L  I  L  I   G  E  T   I  K  I
        R  P  A   S  S  S   .  G  R  P   S  R  L
1121  GTCATCGAGG AGTACGTGCA GCAGCTGAGT GGCTATTTCC
        H  R  G   V  R  A   A  A  E  W   L  F  P
      V  I  E  E   Y  V  Q   Q  L  S   G  Y  F  L
       S  S  R   S  T  C  S   S  .  V   A  I  S
1161  TGCAGCTGAA ATTTGACCCA GAGCTGCTGT TCGGTGTCCA
       A  A  E   I  .  P  R   A  A  V   R  C  P
        Q  L  K   F  D  P   E  L  L  F   G  V  Q
      C  S  .  N   L  T  Q   S  C  C   S  V  S  S
1201  GTTCCAATAC CGCAACCGCA TTGCCATGGA GTTCAACCAT
      V  P  I  P   Q  P  H   C  H  G   V  Q  P  S
       F  Q  Y   R  N  R  I   A  M  E   F  N  H
        S  N  T   A  T  A   L  P  W  S   S  T  I
1241  CTCTACCACT GGCACCCCCT CATGCCTGAC TCCTTCAAGG
        L  P  L   A  P  P   H  A  .  L   L  Q  G
      L  Y  H  W   H  P  L   M  P  D   S  F  K  V
       S  T  T   G  T  P  S   C  L  T   P  S  R
1281  TGGGCTCCCA GGAGTACAGC TACGAGCAGT TCTTGTTCAA
       G  L  P   G  V  Q  L   R  A  V   L  V  Q
        G  S  Q   E  Y  S   Y  E  Q  F   L  F  N
      W  A  P  R   S  T  A   T  S  S   S  C  S  T
1321  CACCTCCATG TTGGTGGACT ATGGGGTTGA GGCCCTGGTG
      H  L  H  V   G  G  L   W  G  .   G  P  G  G
       T  S  M   L  V  D  Y   G  V  E   A  L  V
        P  P  C   W  W  T   M  G  L  R   P  W  W
1361  GATGCCTTCT CTCGCCAGAT TGCTGGCCGG ATCGGTGGGG
        C  L  L   S  P  D   C  W  P  D   R  W  G
      D  A  F  S   R  Q  I   A  G  R   I  G  G  G
       M  P  S   L  A  R  L   L  A  G   S  V  G
1401  GCAGGAACAT GGACCACCAC ATCCTGCATG TGGCTGTGGA
       Q  E  H   G  P  P  H   P  A  C   G  C  G
        R  N  M   D  H  H   I  L  H  V   A  V  D
      A  G  T  W   T  T  T   S  C  M   W  L  W  M
```

FIG. 20D

hCOX-3(con-p)

```
1441 TGTCATCAGG GAGTCTCGGG AGATGCGGCT GCAGCCCTTC
     C H Q G  V S G  D A A  A A L Q
      V I R  E S R E  M R L  Q P F
       S S G  S L G  R C G C  S P S
1481 AATGAGTACC GCAAGAGGTT TGGCATGAAA CCCTACACCT
       . V P  Q E V  W H E T  L H L
     N E Y R  K R F  G M K  P Y T S
      M S T  A R G L  A . N  P T P
1521 CCTTCCAGGA GCTCGTAGGA GAGAAGGAGA TGGCAGCAGA
      L P G  A R R R  E G D  G S R
       F Q E  L V G  E K E M  A A E
     P S R S  S . E  R R R  W Q Q S
1561 GTTGGAGGAA TTGTATGGAG ACATTGATGC GTTGGAGTTC
     V G G I  V W R  H . C  V G V L
      L E E  L Y G D  I D A  L E F
       W R N  C M E  T L M R  W S S
1601 TACCCTGGAC TGCTTCTTGA AAAGTGCCAT CCAAACTCTA
       P W T  A S .  K V P S  K L Y
     Y P G L  L L E  K C H  P N S I
      T L D  C F L K  S A I  Q T L
1641 TCTTTGGGGA GAGTATGATA GAGATTGGGG CTCCCTTTTC
      L W G  E Y D R  D W G  S L F
       F G E  S M I  E I G A  P F S
     S L G R  V . .  R L G  L P F P
1681 CCTCAAGGGT CTCCTAGGGA ATCCCATCTG TTCTCCGGAG
     P Q G S  P R E  S H L  F S G V
      L K G  L L G N  P I C  S P E
       S R V  S . G  I P S V  L R S
1721 TACTGGAAGC CGAGCACATT TGGCGGCGAG GTGGGCTTTA
      L E A  E H I  W R R G  G L .
     Y W K P  S T F  G G E  V G F N
      T G S  R A H L  A A R  W A L
1761 ACATTGTCAA GACGGCCACA CTGAAGAAGC TGGTCTGCCT
      H C Q  D G H T  E E A  G L P
       I V K  T A T  L K K L  V C L
     T L S R  R P H  . R S  W S A S
```

FIG. 20E

hCOX-3(con-p)

```
1801  CAACACCAAG ACCTGTCCCT ACGTTTCCTT CCGTGTGCCG
      Q  H  Q  D   L  S  L   R  F  L   P  C  A  G
       N  T  K   T  C  P  Y   V  S  F   R  V  P
        T  P  R   P  V  P   T  F  P  S   V  C  R
1841  GATGCCAGTC AGGATGATGG GCCTGCTGTG GAGCGACCAT
        C  Q  S   G  .  W   A  C  C  G   A  T  I
      D  A  S  Q   D  D  G   P  A  V   E  R  P  S
       M  P  V   R  M  M  G   L  L  W   S  D  H
1881  CCACAGAGCT CTGA
       H  R  A   L
        T  E  L   .
      P  Q  S  S   D
```

FIG. 20F

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL CYCLOOXYGENASE VARIANTS AND METHODS OF USE

(57) Abstract: The invention relates to the isolation of novel cyclooxygenase type 1 (COX-1) variant enzymes. More specifically, the invention relates to the identification of cyclooxygenase transcripts harboring inton 1, or fragment thereof, of cyclooxygenase 1. The invention further relates to the diagnosis of aberrant cyclooxygenase type 1 variant gene or gene product; the identification, production, and use of compounds which modulate cyclooxygenase type 1 variant gene expression or the activity of the cyclooxygenase type 1 variant gene product including but not limited to nucleic acid encoding cyclooxygenase type 1 variants and homologues, analogues, and deletions thereof, as well as antisense, ribozyme, triple helix, antibody, and polypeptide molecules as well as small inorganic molecules; and pharmaceutical formulations and routes of administration for such compounds.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/30947

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 9/02, 1/20, 15/00; C12P 21/04
US CL : 435/189, 252.3, 320.1, 440, 71.1; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/189, 252.3, 320.1, 440, 71.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
U.S. patent database, est, stremble, pir, swissprot, stn/caplus, EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VOGIAGIS. D., et al., Cyclooxygenase-1 and an alternatively spliced mRNA in the rat stomach: effects of aging and ulcers. Am J Physiol Gastrointest Liver Physiol. May 2000 May, Vol 278, No. 5, pagesG820-7.	1-4 and 14-17
X	FUNK. C.D., et al. P23219. NCBI, 1991, see entire document.	9-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
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15 JUN 2004

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INTERNATIONAL SEARCH REPORT	International application No. PCT/US02/30947

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-17

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US02/30947

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-17, drawn to a nucleic acid encoding COX-1 of SEQ ID NO:2, 5, 14, 15 and 16, vector comprising said DNA, host cell comprising said DNA and a method of producing said protein.

Group II, claim(s) 18-20, drawn to COX-1 of SEQ ID NO:2, 5, 14, 15 and 16.

Group III, claim(s) 21, drawn to an antibody against the protein of Group II.

Group IV, claim(s) 22-24, drawn to a method of detecting the presence of a polypeptide using the protein of Group II.

Group V, claim(s) 25-27, drawn to a method of detecting the presence of a nucleic acid using the nucleic acid of Group I.

Group VI, claim(s) 28-29 drawn to a method of identifying a compound that binds to the protein of Group II.

Group VII, claim(s) 30, drawn to a method of modulating the protein of Group II.

Group VIII, claim(s) 31 and 44-51, drawn to a method of identifying a compound that modulates the activity of the protein of Group II.

Group IX, claim(s) 32-33, drawn to a method of preventing or treating Alzheimer's Disease using an inhibitor of the protein of Group II.

Group X, claim(s) 34-35, drawn to a method of ameliorating a neurodegenerative condition in a subject using an inhibitor of the protein of Group II.

Group XI, claim(s) 36-37, drawn to a method of treating immune or inflammatory condition using an inhibitor of the protein of Group II.

Group XII, claim(s) 38-39, drawn to a method of modulating expression, production or formation of amyloid precursor protein in a subject using an antagonist that is a inhibitor of the protein of Group II.

Group XIII, claim(s) 40-43, drawn to a method of inhibiting COX-3 or PCOX-1 activity using a compound that inhibits the protein of Group II.

Group XIV, claim(s) 52, drawn to a method of making a compound that modulates the activity of the protein of Group II.

Group XV, claim(s) 53-55, drawn to an article of manufacture comprising a compound that modulates the activity of the protein of Group II.

Group XVI, claim(s) 56-63 and 65, drawn to an array of probes that binds to the nucleic acid of Group I.

Group XVII, claim(s) 64 and 66, drawn to a method of choosing therapy for a subject using the probe of Group XVI.

The inventions listed as Groups I-VI Ido not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking Groups I-VI appears to be that they all relate to a cyclooxygenase-1.

However, Vogiagis et al. teach a nucleic acid encoding a cyclooxygenase-1 (abstract).

Therefore, the technical feature linking the inventions of Groups I-XVII does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of Group I is a nucleic acid encoding COX-1 of SEQ ID NO:2, 5, 14, 15 and 16, vector comprising said nucleic acid, host cell comprising said nucleic acid and a method of producing said protein.

The special technical feature of Group II is a COX-1 of SEQ ID NO:2, 5, 14, 15 and 16.

The special technical feature of Group III is an antibody against the protein of Group II.

The special technical feature of Group IV is a method of detecting the presence of a polypeptide using the protein of Group II.

The special technical feature of Group V is a method of detecting the presence of a nucleic acid using the nucleic acid of Group I.

The special technical feature of Group VI is a method of identifying a compound that binds to the protein of Group II.

The special technical feature of Group VII is a method of modulating the protein of Group II.

The special technical feature of Group VIII is a method of identifying a compound that modulates the activity of the protein of Group II.

The special technical feature of Group IX is a method of preventing or treating Alzheimer's Disease using an inhibitor of the protein of Group II.

The special technical feature of Group X is a method of ameliorating a neurodegenerative condition in a subject using an inhibitor of the protein of Group II.

The special technical feature of Group XI is a method of treating immune or inflammatory condition using an inhibitor of the protein of Group II.

The special technical feature of Group XII is a method of modulating expression, production or formation of amyloid precursor protein in a subject using an antagonist that is a inhibitor of the protein of Group II.

The special technical feature of Group XIII is a method of inhibiting COX-3 or PCOX-1 activity using a compound that inhibits the protein of Group II.

The special technical feature of Group XIV is a method of making a compound that modulates the activity of the protein of Group II.

The special technical feature of Group XV is an article of manufacture comprising a compound that modulates the activity of the protein of Group II.

The special technical feature of Group XVI is an array of probes that binds to the nucleic acid of Group I.

The special technical feature of Group XVII is a method of choosing therapy for a subject using the probe of Group XVI.

Accordingly, Groups I-XVII are not so linked by the same or a corresponding special technical feature as to form a single general inventive concept.